TOXICOLOGICAL APPROACHES TO THE METABOLITES OF FUSARIA. XIII.
HEMATOLOGICAL CHANGES IN MICE BY A SINGLE AND REPEATED ADMINISTRATIONS OF TRICHOTHECENES

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Abstract—A single or repeated administration of trichothecene mycotoxins to mice induced leukocytosis and leukopenia. Acute and sub-acute toxicosis of trichothecenes was characterized by hematological and pathological observations.

Key words: trichothecenes, mycotoxins, Fusarium species, leukocytosis, leukopenia,

INTRODUCTION

Fungi belonging to Fusarium species produce spoilage of grains and these molds have been isolated from up to 80% of barley samples collected in one year crop in the southern area of Japan (Tsunoda et al., 1968). Our microbial and chemical surveys have detected the following three kinds of mycotoxins in the metabolites of Fusarium species which were isolated from domestic and imported cereals and grains: uterotrophic zearalenone from F. graminearum (Ishii et al. 1974);
blood-poisonous acetamide (butenolide) from *F. sporotrichioides* (Ueno et al., 1972 b) and cytotoxic trichothecenes (T-2 toxin, nivalenol, fusarenon-X, neosolaniol etc.) from *F. solani*. *F. nivale*, *F. tricinctum et al.* (Ueno et al., 1972 a and 1973 b).

Our previous toxicological studies (Ueno, 1973; Saito and Ohtsubo, 1974; Ueno, 1977 a and 1977 b) reached the conclusion that the trichothecene mycotoxins might be a common toxicant for outbreaks of the following food-borne intoxications in human and farm animals in the world: Akakabi (red-mold)-disease in Japan, alimentary toxic aleukia (ATA) in U.S.S.R., stachybotryotoxicosis in central Europe, and moldy corn toxicosis in U.S.A.

In addition, the authors have demonstrated that repeated administration of T-2 toxin, one of the trichothecene mycotoxins, causes a marked decrease in the circulating white blood cells (WBC) in cats (Sato et al., 1975), and this evidence also supports an assumption that severe leukopenia in the ATA may be caused by the ingestion of toxic trichothecenes.

To disclose the cause of leukopenia by the trichothecene mycotoxins, hematological observations in mice were made with T-2 toxin and related trichothecenes (Fig. 1). The results revealed that a single administration of the trichothecenes caused a marked increase in the number of circulating white blood cells shortly after the administration, and repeated administrations induced decreases in the white blood cells as well as platelets.

**MATERIALS AND METHODS**

The following trichothecenes were isolated from the culture filtrates of *Fusarium* spp. according to the methods previously reported: T-2 toxin and neosolaniol from *F. solani* (Ueno et al., 1972 a), and fusarenon-X from *F. nivale* (Ueno et al., 1971 a). T-2 toxin and neosolaniol were dissolved in olive oil, and fusarenon-X was dissolved in 0.9% NaCl solution. They were administered i.p. or p.o. to male 6-week old ddYS mice. Blood of all mice in series of experiments was collected at 10-11 a.m. from the jugular vein of the unanesthesized mice by using heparinized
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syringes. In the platelet counting, Anticlot/ET (Clinton Lab., Santamonica, Calif., U.S.A.) was used. Numbers of WBC, and red blood cells (RBC) were counted by using Automatic Microcell Counter Model CC-1002, and platelets were counted by using Automatic Platelet Counter Model PL-100 (Toa Medical Technology LTD, Tokyo). Total protein and each protein fraction of the plasma of mice were determined by using Atago Fefractometer and acetate-cellulose electrophoresis apparatus (Gelman Instrument Comp.).

Urea nitrogen (UN), alkaline phosphatase (AL), glutamate-pyruvate transaminase (GTP) and chlorine ion (Cl) were measured by using routine analytical methods. Thrombelastogramm (TEGm) was estimated by using Thrombelastograph apparatus (Hellige Comp., Germany) according to the method of Hartert (Hartert, 1951). Eight to 10 mice were used for each determination.

As for the estimation of feed consumption, each mouse was housed in an individual cage and the difference between the feed given and the residual amount was estimated with desired intervals.

RESULTS

(A) A single administration of trichothecenes

(1) Changes of blood cell counts:

Male mice were administered i.p. with fusarenon-X at three doses of 0.5, 2.5 and 5 mg/kg, and the number of WBC was counted 1, 3, 6 and 24 hours after the administration. In case of the middle dose (2.5mg/kg), the counting continued an additional one week after the injection. As shown in Fig. 2-(A), the total WBC counts increased within one hour after the administration of 2.5 or 5 mg/kg of fusarenon-X, and the elevated level continued for 6 hours and returned to the normal level after 24 hours. The maximum values obtained with 2.5 and 5 mg/kg of fusarenon-X reached 3 and 4 times higher than the control, respectively. The WBC level of the mice treated with 2.5 mg/kg of fusarenon-X ranged in the normal level for the following one week (data not presented).

The i.p. administration of neosolaniol in three doses of 5, 11 and 20 mg/kg caused a similar increase in the number of WBC, and the highest counts which were 3, 4 and 5 times higher than the control, were observed 3-6 hours after the administration, as shown in Fig. 2-(B).

Time-course changes of the leucocyte components revealed that the increase in the number of lymphocytes was more marked and rapid, while neutrophile counts increased later, in the mice administered i.p. with 2.5 mg/kg of fusarenon-X or 11.0 mg/kg of neosolaniol, as shown in Fig. 3-(A) and (B).

All of monocytes, baso- and eosinophilic leucocytes were below 8 % of the total counts at every point. Undiagnosed injured cells with pyknosis and karyorrhexis were at the most 1.4% in compared to 0.06% of the control at 3 hours.
after the administration (data not presented).

Effect of a single administration of fusarenon-X (2.5 mg/kg) or neosolaniol (11.0 mg/kg) on the circulating reticulocytes was summarized in Fig. 4. Temporal increase followed by a marked decrease in the later phase was observed in the neosolaniol-treated mice. This leukemoid-like reaction was not influenced by the preceding splenectomy (unpublished data).

The temporal dose-related elevation of the WBC counts was also observed in mice which received 2.5 mg/kg intraperitoneally or 5 mg/kg orally of T-2 toxin (Fig. 5). In both cases, the maximum elevation was observed 3 hours after the administration.

As for the RBC counts of mice administered i. p. with 2.5 mg/kg of fusarenon-X, no significant change was observed during the 24 hours period (Fig. 6-(A)) while the platelet counts were decreased by 90 and 70% of the control level at one and 24 hours after the administration. This later decline was statistically significant (p<0.05). Oral administration of T-2 toxin in dose of 5 mg/kg caused also a marked decrease of platelet counts after 48 hours (Fig. 6-(B)).

Fig. 2. Changes in white blood cell counts in mice after the trichothecenes. Male ddYS mice (6 week-old) were i. p. administered with (A) fusarenon-X and (B) neosolaniol. Each point in the figures represents an average and S. D. of 10 mice. Number in the figures represents the dose (mg/kg) of trichothecenes.
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(A) Fusarenon-X (i.p. 2.5mg/Kg)

- Lymphocytes
  - Contr.
  - 1 hr
  - 6 hr
  - 12 hr
  - 24 hr
  - 48 hr
  - 72 hr
  - 168 hr

- Neutrophiles
  - Contr.
  - 1 hr
  - 6 hr
  - 12 hr
  - 24 hr
  - 48 hr
  - 72 hr
  - 168 hr

(B) Neosolaniol (i.p. 11.0mg/Kg)

- Lymphocytes
  - Contr.
  - 0 hr
  - 1 hr
  - 3 hr
  - 6 hr
  - 12 hr
  - 24 hr
  - 48 hr
  - 72 hr

- Neutrophiles
  - Contr.
  - 0 hr
  - 1 hr
  - 3 hr
  - 6 hr
  - 12 hr
  - 24 hr
  - 48 hr
  - 72 hr

Fig. 3. Effects of trichothecenes on leucocyte components in mice. (A) 2.5mg/kg of fusarenon-X and (B) 11.0mg/kg of neosolaniol were i.p. administered to male ddYS mice.

Fig. 4. Effects of a single administration of trichothecenes on the circulating reticulocyte counts.

- 2.5mg/kg of fusarenon-X, i.p.
- 11.0mg/kg of neosolaniol, i.p.

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Fig. 5. Changes in white blood cell counts in mice after T-2 toxin. Male ddYS mice (6 week-old) were administered with T-2 toxin in doses of (A) 2.5mg/kg i.p. and (B) 5.0mg/kg p.o. Each point represents an average and S.D. of 8 mice.

Fig. 6. Changes in red blood cells and platelet counts and total plasma protein in mice after the trichothecene administration. Male ddYS mice (6 week-old) were administered with (A) 2.5mg/kg of fusarenon-X i.p. or (B) 5mg/kg of T-2 toxin p.o. Each point represents an average and S.D. of 8 mice.

O---O RBC; ●●● platelet; ■■■ total protein.
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(2) Changes of plasma protein and components:
The total plasma protein of mice administered with fusarenon-X (i.p. 2.5mg/kg) or T-2 toxin (p.o. 5.0mg/kg) were not changed (Fig. 6). The changes of serum protein fractions in mice administered with i.p. 2.5mg/kg of fusarenon-X or 11.0mg/kg of neosolaniol were investigated with a result that the decrease in the ratio of albumin and γ-globulin and concomitant increase of β-globulin were notable 48 hours after the administration (data not presented).

Several serum enzymes and chemical components were determined in the mice which received 2.5mg/kg of fusarenon-X or 11.0mg/kg of neosolaniol. As shown in Fig. 7, GTP, AL and Cl were not markedly changed during 72 hours of the observation period. Statistically significant change was the decrease of UN. Fusarenon-X caused a decrease (60% of control) at the 6th hour after the administration, and neosolaniol induced a marked decline (below 50% of control) after 12 hours of the administration.

(3) Changes of organ weights:
The single i.p. administration of 2.5mg/kg of fusarenon-X caused no significant changes in body weight, absolute or relative weights of the liver, kidney, spleen and brain (data not presented). In the mice received i.p. 11mg/kg of neosolaniol a notable decrease in the relative weight of spleen was induced 3-48 hours after the administration (Fig. 8). and it was recovered 72 hours later. Other organs such as liver, kidney, heart and brain were not affected by the toxin.

(4) Pathological findings
A time course study carried out in mice with a sublethal dose of neosolaniol (i.p. 11.0mg/kg) revealed that the small intestine was the earlist organ to be affected. In a half to one hour after the i.p. injection the cells of the crypts showed swelling of the nuclei and the mitotic figures have disappeared. Then crypt cell necrosis followed in 3 hours, reaching its maximum after 6 hours. As early as 24 hours the cellular debris in the crypts was apparently cleaned off and reepithelisation began. At this time, the villi lost their tall, thin figures and became short and edematous. The epithelium regenerated completely on the 3rd day. The similar changes were observed in the colon and stomach but with lesser severity.

In the lymphoid tissues karyorrhexis was most prominent during 3 to 6 hours in the secondary follicles of the lymph nodes, 6 to 12 hours in the follicles of the spleen and in 6 to 24 hours in the cortex of the thymus, leading to follicular or cortical atrophy up to the 3rd day. In the spleen lymph follicles became smaller in the early phase (0.5 to 6 hours), later the red pulp became more atrophic (1-3 days). In the other organs there were little pathologic changes with this level of intoxication.

After the i.p. injection of 2.5 mg/kg of fusarenon-X, pathological changes were similar to, but not so severe as those occurring after neosolaniol administration.

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Fig. 7. Changes of plasma enzymes and components in mice by fusarenon-X and neosolaniol. Male ddYS mice (6 week-old) were i.p. received (A) 2.5mg/kg of fusarenon-X, or (B) 11mg/kg of neosolaniol. Each point is an average and S.D. of 10 mice.

- - - - - - GPT: ■ ■ ■ AL: ○ ○ ○ UN: × × × Chloride ion.
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Fig. 8. Effects of neosolaniol on the body and organ weights in mice. ddYS mice were i.p. administered with 11mg/kg of neosolaniol, and sacrificed at the time indicated. Each point represents an average and S.D. of 10 mice.
(B) Sub-acute toxicity of trichothecenes in mice

(1) Selection of sub-acute doses of trichothecenes

In order to evaluate the sub-acute toxicity of trichothecenes, mice were administered orally varied doses of T-2 toxin and fusarenon-X daily for 1-3 weeks and the incidence of fatal cases and the changes in body weight were estimated.

T-2 toxin was administered daily to mice in doses of 1.5, 3.0, 6.0 and 9.0 mg/kg orally. Each group was consisted of 10 mice, and the lethality was observed for three weeks, as shown in Fig. 9-(A). No fatal case was observed at the dose of 1.5mg/kg during 20 days of intubation. While, all the mice died at the dose of 3.0mg/kg after a daily intubation for 15 days. High doses of 6 and 9mg/kg caused 100% lethality within 4 days. Intubation of fusarenon-X in daily dose of p.o. 1.5mg/kg caused no fatal case for 28 days, and with the daily doses of 3.0 and 5.0mg/kg, all test mice died within 9 and 5 days, respectively (Fig. 9-(B)). The body weight of mice administered with a high dose over 3mg/kg of T-2 toxin and fusarenon-X was reduced in compared with the control (data were not shown).

In another experiment with the pellet which contained various doses of fusarenon-X, a daily uptake of fusarenon-X in the dose of 3.0mg/kg caused no fatal case even after 30 days, and with 6.3mg/kg, about a half of the total died within 28 th day of feeding (Fig. 9-(B)).

(2) Effects of T-2 toxin on feed consumption

Since the single and repeated administrations of trichothece compounds caused an increase of fatal cases as above and various changes in blood cell counts, contents of plasma components as described below, it was aimed to investigate whether these toxicological effects were originated from direct effects of toxic agents or indirect ones through a reduced feed consumption. In this respect, each mouse was housed individually and the feed consumption was estimated by measuring the difference between the initial amount of feed given and the residual at the desired time after the trichothecenes. Fig. 10 represents the cumulative feed consumption curves of the control mice and the treated mice p.o. administered with a single dose of 5mg/kg of T-2 toxin or with the daily 3mg/kg of T-2 toxin for 5 days. Average uptake was estimated to be 5g/day/mouse in the control.

In the case of single shot of T-2 toxin, the feed consumption was reduced to the half of the control on 14-24 hours after the administration. At initial 6 hours and the later 24-48 hours no depression was shown in the feed uptake (Fig. 10-(A)). In the case of daily exposure of mice for 5 days, the daily feed consumption was reduced to around a half of the control value.

(3) Changes of blood cell counts and plasma enzymes in sub-acute toxicosis by T-2 toxin

A hematological changes in mice which received a daily dose of 3.0mg/kg of T-2 toxin for 3 and 5 days and were sacrificed 24 hours after the last intubation
Leukopenia in Mice by Trichothecces were shown in Fig. 11 and Table 1. The red blood cell counts were within a normal range, while WBC was reduced from 3 200/mm³ (control) to 2 400/mm³ (3 days) and 700/mm³ (5 days). These values are 75 and 22% of the control, respectively. Platelet counts were also reduced to 38 and 21% of the control, respectively.

![Fig. 9](image-url) Survival curves of mice given orally various doses of T-2 toxin or fusarenon-X. Male ddYS mice (6-week old) were administered daily (A) an olive oil containing T-2 toxin or (B) a saline containing fusarenon-X. In Exp. (B), the mice were fed with the diet containing fusarenon-X. Each group consists of 10 mice.
Fig. 10. Effects of a single and repeated administrations of T-2 toxin on feed consumption in mice.
(A) 5.0 mg/kg of T-2 toxin was singly intubated at 10 a.m.;
(B) 3.0 mg/kg of T-2 toxin was daily intubated for 5 days at 10 a.m.;
Cumulative feed consumption was expressed as g/mouse±S.D.
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**ddYS male mice (6 week-old)**

T-2 toxin 3 mg/kg/day p.o.

**Fig. 11.** Effects of repeated administration of T-2 toxin on blood cells counts and plasma protein contents. ddYS male mice were p.o. administered with 3 mg/kg of T-2 toxin for 3 and 5 days.

- **plasma protein**;
- **WBC**;
- **RBC**;
- **platelets**;

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Further experiments with a daily 2mg/kg of T-2 toxin revealed that, as shown in Fig. 12, the intubation for 3 weeks resulted in the marked decline of WBC and platelet counts, but the RBC counts and Hb content (data not shown) remained in the control level. Termination of T-2 toxin intubation resulted in a

![Graph showing changes in blood cells and plasma protein by T-2 toxin. Male ddYS mice (6 week-old) were daily intubated for 3 weeks with the toxin dissolved in olive oil in a dose of 2.0 mg/kg. Each point is an average and S.D. of 8 mice.](image-url)
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Table 1. Hematological observations in mice following repeated administration of T-2 toxin.

<table>
<thead>
<tr>
<th>T-2 toxin (mg/kg × days)</th>
<th>No. of mice</th>
<th>Total protein (g/dl)</th>
<th>Protein fractions (%)</th>
<th>A/G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alb.</td>
<td>$\alpha_1$-Glb.</td>
</tr>
<tr>
<td>0</td>
<td>6</td>
<td>5.9±0.4</td>
<td>52.8±2.2 7.9±0.7 16.5±1.5 11.7±1.3 11.1±1.0 1.12±0.10</td>
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</tr>
<tr>
<td>3×3</td>
<td>5</td>
<td>5.3±0.6</td>
<td>47.5±4.6 7.9±0.6 17.5±1.0 12.7±1.5 14.4±3.2 0.90±0.18*</td>
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</tr>
<tr>
<td>0×</td>
<td>6</td>
<td>5.8±0.2</td>
<td>51.7±2.1 9.7±0.9 15.6±1.1 11.8±1.3 11.2±0.9 1.07±0.09</td>
<td></td>
</tr>
<tr>
<td>3×5</td>
<td>4</td>
<td>5.6±0.3</td>
<td>50.6±3.7 7.6±1.2 16.4±0.6 13.0±1.6 12.4±1.5 1.02±0.16</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Thrombelastogram values in mice following repeated administration of T-2 toxin.

<table>
<thead>
<tr>
<th>T-2 toxin (mg/kg × day)</th>
<th>No. of mice</th>
<th>TEG values a) (r K r+K ma) (min)</th>
<th>platelet (10^5/mm^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>6.92±0.47 1.42±0.13 8.33±0.53 73±4</td>
<td>8.39±0.77</td>
</tr>
<tr>
<td>3×3</td>
<td>5</td>
<td>7.05±0.37 1.65±0.22* 8.70±0.45 75±3</td>
<td>4.39±0.97**</td>
</tr>
<tr>
<td>0</td>
<td>6</td>
<td>7.08±0.75 1.58±0.20 8.67±0.73 74±3</td>
<td>8.47±0.98</td>
</tr>
<tr>
<td>3×5</td>
<td>5</td>
<td>10.15±0.97** 3.20±0.78* 13.35±1.07** 69±3</td>
<td>3.17±1.46**</td>
</tr>
</tbody>
</table>

a) r, reaction time; K, coagulation time; ma, maximum amplitude.
* P<0.05; ** P<0.01.

Table 3. Effects of repeated administrations of T-2 toxin on the plasma protein fractions.

<table>
<thead>
<tr>
<th>T-2 toxin (mg/kg × days)</th>
<th>No. of mice</th>
<th>RBC (10^6/mm^3)</th>
<th>WBC (10^3/mm^3)</th>
<th>Platelets (10^3/mm^3)</th>
<th>Hb (g/dl)</th>
<th>Ht (%)</th>
<th>Total Plasma protein (g/dl)</th>
<th>s-GOT (Karmen unit/ml)</th>
<th>GPT (mU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>8.0±0.3</td>
<td>3.2±0.8</td>
<td>7.7±0.9</td>
<td>14.6±0.6</td>
<td>47±2</td>
<td>6.0±0.4</td>
<td>50±6</td>
<td>0.6±0.8</td>
</tr>
<tr>
<td>3×3</td>
<td>6</td>
<td>6.6±0.5**</td>
<td>2.4±0.9</td>
<td>3.0±1.9</td>
<td>12.5±1.0</td>
<td>38±4</td>
<td>5.4±0.4</td>
<td>73±16</td>
<td>—</td>
</tr>
<tr>
<td>3×5</td>
<td>7</td>
<td>7.4±1.2</td>
<td>0.7±0.5**</td>
<td>1.6±0.6</td>
<td>14.0±2.0</td>
<td>43±8</td>
<td>5.4±0.5</td>
<td>70±28</td>
<td>0.7±0.6</td>
</tr>
</tbody>
</table>

T-2 toxin dissolved in olive oil was p.o. administered.
• P<0.05; ** P<0.01.
recovery in WBC and platelet counts.

(4) Effect on thrombelastogramm:

Daily administration of 3mg/kg of T-2 toxin for 5 days caused increments in reaction time (r), coagulation time (K) and consequently (r+K) value. The repeated 3 days intubation resulted in only an elongation of K value, as summerized in Table 2. Positive control experiment with warfarin, which is a potent rodenticide and possesses a potent anticoagulant activity, revealed a marked reduction of platelet counts and an increase of (r) value infinitively (data were not presented).

![Graph showing effects of T-2 toxin on organ weights in mice.](image)

**Fig. 13.** Effects of T-2 toxin on the organ weights in mice. T-2 toxin was p.o. administered in dose of 3mg/kg for 3 and 5 days. Each point is an average and S.D. of 4-8 mice.

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(5) Effects of repeated administration of T-2 toxin on the plasma protein fractions:
Table 3 summarized the data on the total plasma protein as well as the protein fractions in mice received p.o. 3 mg/kg/day of T-2 toxin for 3 and 5 days. In general, no marked alteration in the total and each plasma protein was observed.

(6) Changes of organ weights after T-2 toxin
As for the organ weight of mice administered with p.o. 3 mg/kg of T-2 toxin for 3 and 5 days, a marked reduction was observed with the thymus and spleen and no changes were detected with the liver, kidneys and others, as shown in Fig. 13. Noticeable change was the thymus, in which the relative weight was reduced to 39 and 17% of the control 3 and 5 days after the T-2 toxin intubation.

DISCUSSION

Saito et al. (1969) observed an immediate elevation in level of WBC counts in mice which received a crude toxin from F. nivale, which produces the trichothecces such as nivalenol and fusarenon-X. The present data also revealed that the three kinds of trichothecces caused a temporary leukocytosis in mice shortly after the i.p. and p.o. administrations.

The i.p. LD_{50} values (mg/kg) to the same aged male mice were estimated as 3.4 (fusarenon-X), 5.2 (T-2 toxin) and 14.5 (neosolaniol) (Ueno et al., 1973). These values make a ratio of 1.0 : 1.5 : 4.3. The relative potenciality in the temporary leukocytosis of these three trichothecces corresponded to the above relative lethal toxicity.

Because of the different structures in the side chains of these three trichothecces (Fig. 1), T-2 toxin is not soluble to water, fusarenon-X freely soluble, and neosolaniol is situated in the middle of the above two toxins. Irrespective of the different solubility to an aqueous solution, the onset and duration of temporary leukocytosis in mice are similar to each other (Fig. 2 and 5), showing that these three toxins are absorbed with almost equal rapidity. Absorption from the peritoneal cavity is usually quite rapid. Notable findings were that the p.o. administered T-2 toxin exerts its leukocytotic activity rapidly as the case of i.p. administration (Fig. 5).

As to the cause of the temporary leukocytosis by trichothecces, the authors suggest that some local inflammatory action gives rise to one kind of shock which may cause discharge of WBC stored in lymphatic tissues into circulating system, since, as reported in the preceding paper (Ueno et al., 1970a), the trichothecces possess an inflammatory action to skin and mucous membranes and their inflammatory potenciality is in the decreasing order of T-2 toxin > neosolaniol > fusarenon-X.

The i.p. and p.o. administrations of trichothecces caused a decrease of circulating platelets in the later stage of administration (Fig. 6). Since the admin-
istration of trichothecenes caused a reduction in feed consumption (Fig. 10), the authors aimed to analyse whether such malnutrition influences on the circulating platelet counts. However, the number of circulating platelets of mice fed a half amount of the control mice does not differ from that of control mice (unpublished data). Therefore, it is convinced that the decline of platelet counts does not relate with the decreased feed consumption.

As reported in the preceding paper (Saito et al., 1974), the trichothecenes induce cellular damages in the actively-dividing cells of small intestine, thymus, spleen, reproductive tissues and bone marrow, and the present time-course observation have confirmed these pathological injuries. The decrease of circulating platelet counts is therefore presumed to be caused by an impairment of hematopoietic system in the bone marrow.

As for the plasma components, UN was decreased in the later phase of trichothecene intoxication (Fig. 7), and this decline is presumed to be caused by the reduction of feed consumption in the mice received a single shot of trichothecenes.

The single p. o. LD50 values of T-2 toxin and fusarenon-X to male ddYS mice aged 6-week old, were estimated to 7 and 4.5 mg/kg, respectively (Sato and Ueno, 1977). However, as mentioned in the section (B) of the present paper, with a daily dose of 1.5 mg/kg for 20 days, 30 mg/kg in total or 4 LD50 T-2 toxin caused no fatal cases, and with a daily dose of 3 mg/kg for 8 days, 24 mg/kg in total, a half of the mice died (Fig. 9). Similar results were obtained with fusarenon-X; a daily dose of 1.5 mg/kg for 30 days, 45 mg/kg in total or 10 LD50, is safe for mice as far as concerns the lethal toxicity. Furthermore, when the toxin was given as a pellet, a daily feeding of 3 mg/kg for 30 days, 90 mg/kg in total or 20 LD50 caused no fatal cases. Gas chromatographic analysis revealed no degradation of the trichothecenes added to the feed during this experimental period. Therefore, it was convinced that the trichothecene mycotoxins were not cumulatively affecting toxicants to the animals or their excretion to outside of the body was very rapid. Actually, employing a tracer technique it was proved the rapid distribution and elimination of fusarenon-X and T-2 toxin in mice and rats (Ueno et al., 1971 b; Matsumoto et al., 1978).

It should be pointed out that the selection of daily dose of trichothecenes is very important for evaluate a sub-acute as well as chronic toxicity of trichothecene mycotoxins.

Hematologically, the oral administration of T-2 toxin in doses of 3 mg/kg for 3-5 days or 2 mg/kg for 1-3 weeks was proved to depress the counts of WBC as well as platelet counts in mice (Table 1, Figs. 11 and 12). Since it has been established that the blood cells such as WBC, platelets and RBC are originated from common stem cells in bone marrow and that their half life times are 3-5 days,
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4-5 days and 50-60 days, respectively. The rapid decrease of circulating WBC followed by platelets was presumed to follow their life time. From these evidences, the authors convinced that the trichothecene mycotoxins given repeatedly induce a disorder of hematopoietic system of bone marrow to result in the depression of supply in the short-life blood cells such as WBC and platelets. As for RBC, the short-term exposure of mice to T-2 toxin (3 mg/kg for 3-5 days) caused no marked decrease in the circulating number (Table 1 and Fig. 11), and rather a long term test (2 mg/kg for 3 weeks) induced a slight decrease in RBC counts (80% of control) (Fig. 12). Since the half-life time of RBC is much longer than those of the above WBC and platelets, it may be hard to demonstrate a marked reduction of RBC counts under the present experimetal conditions. More precise information on the hematopoietic injury induced by the sub-acute as well as chronic exposure to trichothecene mycotoxins will be expected from the analysis of leucocyte and red blood cell subfractions.

From the evidences that the repeated treatment of mice with the sublethal doses induces a reduction on platelet counts the authors convinced that the clotting time of the blood from the trichothecene-treated mice is much longer than that of control mice. Irrespective of the marked reduction of platelet counts in both treated mice (3 mg/kg for 3 and 5 days), the thrombelastogramm (Table 2) revealed the significant elongation of clotting time was observed only in the later group and the maximum amplitude (ma) fell in the normal range in both cases. Therefore, in the contrast to the case of walfarine, T-2 toxin is not considered to possess the ability to interfere directly the blood coagulation system.

In the preceding papers (Ueno et al., 1968, 1970 b and 1973 a ; Ohtsubo et al., 1970), the authors demonstrated that the trichothecene compounds are a potent inhibitor of protein synthesis in eukaryotic cells in vivo and in vivo. Such biochemical property of the trichothecenes is presumed to contribute the functional damage of hematopoietic system noted above.

As for other parameters which represent characteristic changes in the plasma enzymes and components, no information is available so far. Further detailed analysis on the plasma proteins and chemical components is needed for characterization of the acute and subacute toxicosis induced by the trichothecenes.

The short-term exposure to T-2 toxin resulted in a marked decrease in the weights of thymus and spleen (Fig. 13), and pathologically the marked finding is atrophy of these organs. These findings coincide with the above discussion that the trichothecenes attack selectively the proliferating cells of the tissues (Ohtsubo and Saito, 1977).

SUMMARY

Hematological changes in mice by a single and repeated administration of
three trichothecenes, cytotoxic mycotoxins from fungi belonging to *Fusarium* species, were investigated. A single intraperitoneal or oral administration of T-2 toxin, neosolaniol and fusarenon-X in sublethal doses caused a temporary increase in the number of circulating white blood cells shortly after the administration (1-12 hours). The degree of this leukocytosis in mice was proportional to the doses administered and their relative lethal toxicities. The red blood cell counts were not altered, but the platelet counts were decreased in the later stage of intoxication. Among the serum protein fractions, albumin and γ-globulin were decreased and β-globulin was increased. No marked changes in serum glutamate-pyruvate transaminase and alkaline phosphatase activities, and in the concentration of chloride ion were observed during the experimental period. Urea nitrogen was decreased in the later phase of intoxication. Pathologically, the small intestine was the earlist organ to be damaged, and followed by karyorrhexis in the lymphoid tissues.

In the mice administered orally with a sublethal dose of T-2 toxin or fusarenon-X for 1-3 weeks, marked declines in the circulating white blood cells and platelets with concomitant decrease in the thymus and spleen weights were observed. Neither coagulation system nor plasma protein fractions was altered. Histological examination revealed atrophy in the thymus and spleen.

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**REFERENCES**


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