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HEPATOTOXICITY OF T–2 TOXIN IN RATS

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ABSTRACT: *T–2 toxin was given to rats(Spargue Dawley) with a 4mg/kg and 2mg/kg dose p.o. Data was based on the T–2 toxin 4mg/kg treated group except for counting of acidophil bodies. GPT activities increased significantly from 0.5 to 10 hours. GOT activities were also increased at 1 and 5 hours significantly and the relative weights of liver were increased at 0.5 and 5 hours significantly. There were slight necrotic foci in microscopic observation. There was a dose–dependent trend for the frequency of the acidophil body between the 4mg/kg treated group and the 2mg/kg treated group. The location of the acidophil body was not selective, and they were isolated forms or colony–like forms. In electronmicroscopic observation, round electron-dense materials and myelin–like changes were observed in mitochondria of hepatocyte at 0.5 hour. Acidophil bodies consist of fragmented RERs and other organelles, which are barely recognizable, some acidophil bodies consist of autophagous vacuoles with double(lysosomal) membranes, glycogen granules and vacuole–like inclusions, several of which contain myelin like formation. Band-shape arrangements of RERs were observed for 5 hours, and microvilli of bile canaliculi were intact 5 hours after dosing.*

Keywords: *T–2 toxin. clinical pathology. light microscopy electron microscopy. acidophil body, hepatotoxicity.*

INTRODUCTION

T–2 toxin is one of fungal metabolites produced by various species of *Fusarium, Myrothecium, Trichoderma, Cephalosporium, Verticimonosporium* and *Stachybotrys,* and it was known as one of the most toxic fungal metabolites (Ueno 1977a). This kind of fungus usually contaminated by certain storage states of various agricultural products and seasonal, climate conditions, so domestics and poultry easily happen to be affected by feeding of foodstuffs (Shreeve 1975, Mirocha 1976). Therefore, it is possible to transmit it to humans who consume the animal products. It has been known that various diseases are poisonings originating from trichothecene, as is viewed in outbreaks in nations and animals, and by symptoms. A noteworthy historical report was "Taumelgetreide toxicosis"(Woronin 1891) in the Ussuri district of Siberia In 1942–1947, people in Orenberg were fatally affected by overwintered cereals. This serious intoxication was given the name "Alimentary Toxic Aleukia". And there were many losses in dogs, horses, swine and poultry in addition to the symptom of feed–refusal. In 1931, massive intoxication of horses, swine, calves, and poultry developed in the Ukraine and central Europe. The major symptoms were shock, stomatitis, dermal necrosis, hemorrhages, leukopenia, nervous disorders and death from respiratory failure. In 1958, again in the Ukraine, the illness struck horses and thousands of cattle(Rod-

ricks *et al.* 1974). In 1940–1946, outbreaks of the disease in hens(Sarkisov 1947) and poultry, horses, and swine(Palyusik 1970) were reported in Hungary. As for the stachhbotryotoxicosis, the trichothecene family have been isolated. During the past several decades in the northern Japanese province of Hokkaido, the high disease incidence in horses was observed. The major symptoms of this "bean–hull poisoning of horses" were convulsion, cyclic movement, disturbed respiration, decreased heart rate, and retarded reflexes. And microbiological survey has demonstrated a high degree of contamination of neosolaniol and T–2 toxin (Ueno *et al.* 1972a). Albright et al(1964) reported serious problems in Illinois associated with ingestion of moldy corn by cattle. Analysis showed that the causative toxic agent was T–2 toxin(Bamberg 1968) Ueno(1977b) summarized that this kind of poisonings mostly can be attributed to mycotoxins of the 12–13–epoxytrichothecene family.

Many lesions and effects by T–2 toxin are described as radiomimetic in nature; CNS toxicity resulting in anorexia, lassitude, and nausea; suppression of reproductive organ functin; and acute vascular effects (Sato and Ueno 1977). Moreover, it is necessary for the medical research of T–2 toxin because T–2 toxin has been used as a chemical weapon(Burt 1982, U.S. Depatment of State 1982, Ember 1984).

Many investigators mostly use rats as experimental animal in a lot of nutrition tests, metabolism, and toxicity tests, and would be confused in discrimination of toxicity by the use of mycotoxin contaminated rats. It is necessary for us to confirm the degree of hepatotoxicity of T–2 toxin, so, in this paper, we tried to confirm the hepatotoxicity of T–2 toxin in rats. In previous reports, pathological studies of hepatotoxicity in rats by T–2 toxin were a few, especially electron–microscopic studies. In summary of hepatotoxicity of T–2 toxin in rodents, Schoental and Joffee(1974) in rat and mouse, Schoental and Yagen(1979) in weanling rats, Rukimini(1980) also in weanling rats, and Lee(1984) in mice reported no specific lesions in their microscopic observation. Kosuri reported the observation of focal necrosis in the liver of cattle(1970), and rats(1971) Eugene(1973) also reported the necrotic lesions and hemorrhages in gut, kidney, and liver. A experiment about several exposure route(IP. IM. SC) revealed multiple, randomly spaced foci of coagulative necrosis in rats-(Brennecke et al. 1982). Recently, Chan(1984) reported that the effect of T–2 toxin in liver was selective. Gentry(1984) reported the liver was not primary target organ in cattle. Tsuchida et al.(1983) observed clumping and fading of basophilic masses, of irregular appearance of the masses, minute vacuole at the periphery of hepatocytes, and many hyaline droplets microscopically. And they reported that many hyaline droplets were noticed at the 3rd and 6th hours of the experiment, and markedly decreased in number by the 24th hours. As in the fore–mentioned descriptions, it is important that the previous information of specific lesions according to the consumption of contaminated feedstuffs in various kind of animal tests. Therefore, we tried to find the correlation of acidophil body and hepatotoxicity, or which organelles were primary targets by using of Sprague–Dawley rats, with gross, microscopical and electronmicroscopical observations.

MATERIALS AND METHODS

Experimental Animal; We used 6 week–old Sprague–Dawley male rats from Yuhan Experimental Animal Center, and purchased feedstuffs from Samyang feedstuffs Co. Tap water was supplied ad libitum. The room was maintained at $22\pm4°C$, $65\pm5\%$ with day light photoperiod. All animals were randomly selected groups of five per cage and were housed in polycarbonate cages on soft–wood shavings.

T–2 toxin; T–2 toxin was dissolved in 6.2% ethanol 25 hours prior to dosing. T–2 toxin

2mg/kg b.w. or T–2 toxin 4mg/kg b.w. were treated with a ball–tipped needle connected with syringe intragastrically. Autopsy was performed at 0.5, 1, 5, 10, 15 hours(4mg/kg treated group only), 20 hours and 48 hours(2mg/kg treated group only) after dosing.

Collection of specimen; All animal was fuscinated 12 hours before dosing, and were dissected on abdomen after ether inhalation. Blood was collected from abdominal aorta. Then liver, spleen, kidney and thymus were isolated for fresh weights. Immediately a specimen from the right lobe of the liver for electron microscopy was collected. Several parts of the right, left lobes, and median lobes were dissected longitudinally in order to be easily infiltrated with 10% neutral buffered formalin for light microscopy.

Hematological Examination; Na–EDTA were used as anticoagulants and Coulter counter (Coulter Electronics USA) and counting chambers were used for counting RBC and WBC. The microhematocrit method was used in determination of PCV, and Buiret method in total protein. Collected blood was left standing in a glass test tube at room temperature for 60 min and centrifuged at 1500g for 20 mins in order to obtain serum for enzyme activity studies. The collected serum was stored in a deep freezer.

Pathological Examination; For light microscopical studies, Formalin fixed livers were trimmed to 5mm thick–preparations, 3 pars of the right and left lobes, 2 parts of the median lobes. Therefore, they were washed with running water, and all the tissues were processed by an Automatic tissue Processor (Fisher, Model 166A, U.S.A.) until reaching the stage of paraffin blocks. All blocks were cut to 5–6 μm by Microtome (Am. Op. Model A0 810, U.S.A.), and stained with hematoxylin and eosin, methylene blue–eosin(Variant Stovall black method). Especially, 100 views per preparation were observed with 400X magnification for the size and number of acidophil bodies. For electron microsopical studies, after the isolation of the liver, sampling was performed immediately from the one–third terminal part of the right lobes with the size of 3x5x1 mm^3. After the prefixation with 4°C, 2.5% Glutaraldehyde for 180 min, they were washed with Sörensen buffer (pH 7.2, 4°C) for 10 mins. And trimmed again to 1x1x1 mm cubic size for post fixation (90 min) with O_sO_4 (4°C, 1%). After fixation, serial dehydration was processed. Epon mixture transfered to the well and embedded in the dehydrated specimen. All blocks were dried in over 3 days. After ultrathin (50 70 nm) preparations were sectioned by Ultramicrotome (Sorval MT 5000). They were stained double with uranyl acetate and lead citrate. All samples were observed with JEOl 120CX II electron microscopy, 80KV.

Satistical Methods; Statistics about the size and number of acidophil body was calculated as a simple means as one unit from 8 data of each liver of a rat. Acidophil body, relative weights of organs, and body temperature were used one–way ANOVA in order to compare results in T–2 treated animals and vehicle–treated animals at the same time points. Blood cells and serum enzyme activities were used for the Student–t test. Probability was performed at 95%($P<0.05$) and 99%($P<0.01$).

RESULTS

RBC counts showed transient increase by $8.48\pm0.34\pm10^6$/ml significantly($P<0.05$), and then decreased significantly ($P<0.01$) 15 hrs after dosing. WBC counts showed significant ($P<0.01$, $P<0.05$) increases at 1hr($12.31\pm2.14\times10^3$/ml) and 5hr($7.20\pm0.63\times10^3$/ml), and therefore, showed abrupt falls(Fig. 1). PCVs showed significant ($P<0.05$) decreases by 37.2 ± 4.21% 1 hr after dosing and siginificant ($P<0.01$) increases by 51.0 ± 1.73% 5 hrs after dosing(Fig. 1). Serum total protein showed significant($P<0.05$) decreases by $4.00\pm$ 0.66 mg/dl(10 hrs) and 3.77 ± 0.81 mg/dl(15 hrs) after dosing(Fig. 2).

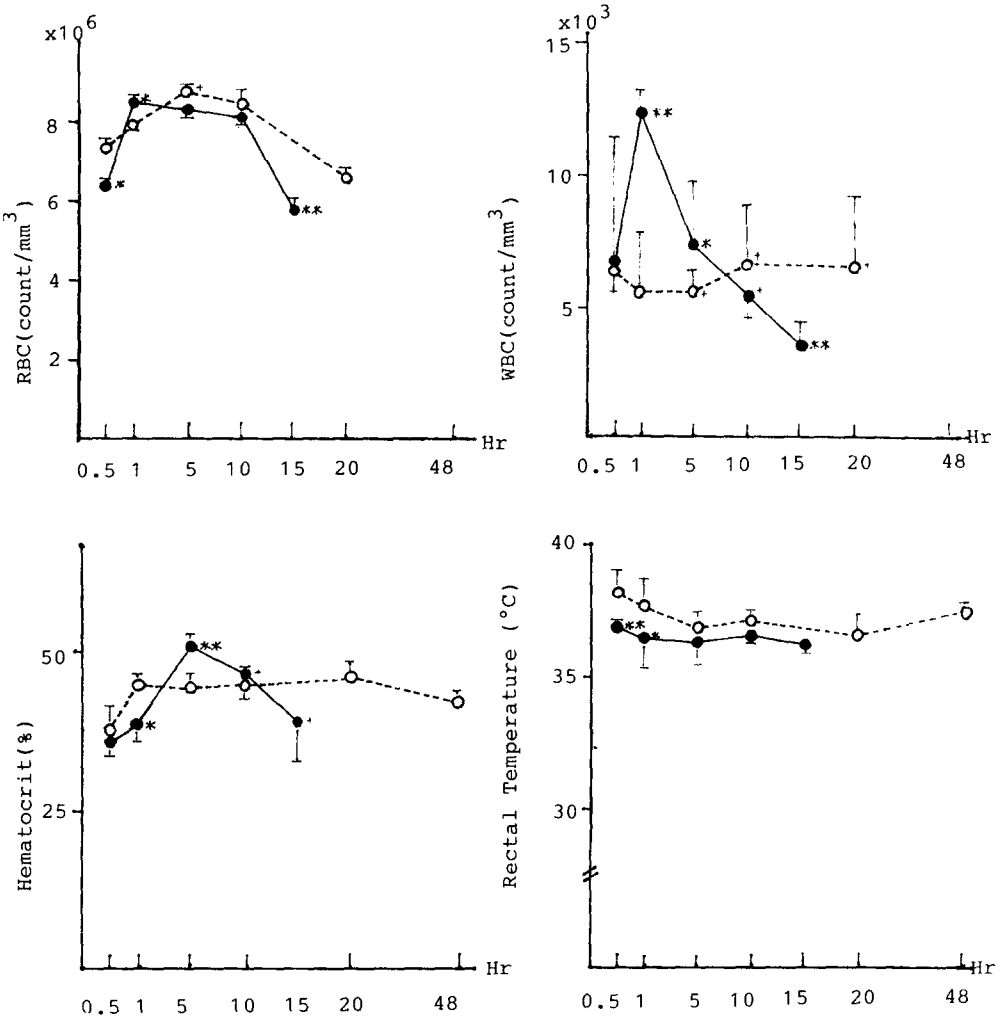


Fig. 1. ○, Control: ●,T-2 toxin. +Data based on three rats. Significantlydifferent from contro:*$P<0.05$; **$P<0.01$. Changes in blood cell counts, hematocrit value and rectal temperature in rats administered T-2 toxin. Animals were treated p.o. 4mg of T-2 toxin/kg body weight. Dissolved in 6.2% EtOH or the vehicle alone. Each value in the figures represents the mean±SD.

GPT activities is the highest as 147.3±62.83 U/L 5 hrs after dosing, and significant($P<0.05$, $P<0.01$, $P<0.05$, $P<0.05$) increases were shown 0.5, 1, 5, and 10 hr after dosing respectively(Fig. 2). GOT activities also showed 138.4±9.67 U/L significantly($P<0.01$) 1 hr and the highest value as 340.9±74.87 U/L 5 hr after dosing. But ALP activities did not show significance during all of the time course study (Fig. 2).

The relative weights of body weight of liver in the T-2 toxin 4mg/kg treated group showed significant($P<0.05$) increase by 4.09±0.67 at 0.5 hrs and also significant ($P<0.05$) increase 5 hrs after dosing. Kidney weights increased significantly ($P<0.05$) by 1.18±0.09 at 0.5 hrs and also increased 5 and 10 hrs significantly ($P<0.01$, $P<0.05$) after dosing.

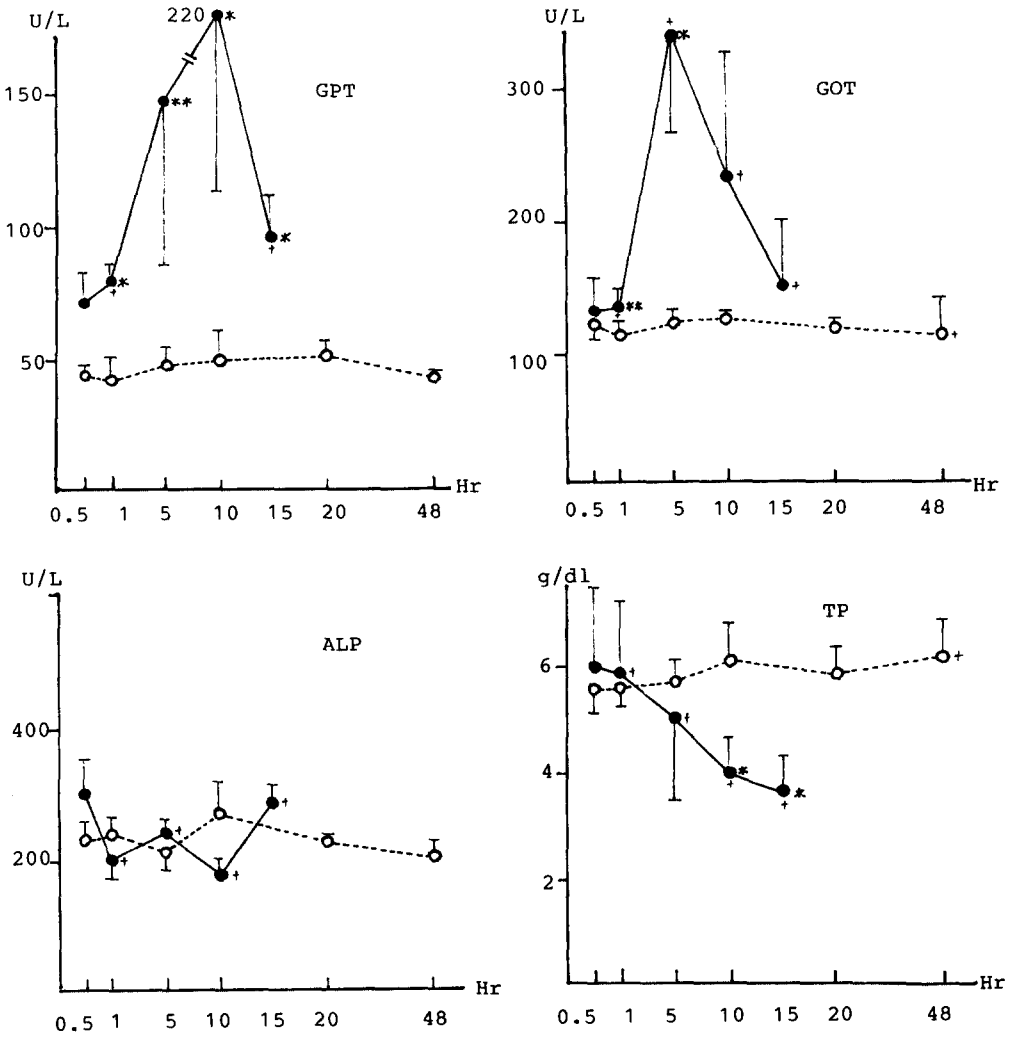


Fig. 2. ○, Control: ●, T–2 toxin. +Data based on two rats. Singificantlydifferent from control:*$P<0.05$; **$P<0.01$. Changes in levels of GPT, GOT, ALP and total protein(TP) in plasma of rats administered T–2 toxin/kg body weight dissolved in 6.2% EtOH or the vehicle alone. Each value in the figures represents the mean ± SD.

Spleen and thymus also increased significantly ($P<0.05$) and/or 1 hr after treatment.(Fig. 3).

The changes of body temperature showed significant ($P<0.01$, $P<0.05$) decreases by 37.0 ± 0.13°C and 36.4 ± 1.03°C 0.5 and 1 hr after dosing respectively(Fig.1). It was the 10 hr group in which the highest frequency of acidophil bodies were revealed in the T–2 toxin 2mg/kg treated group, In 100 views with 100X magnification, acidophil bodies which were larger than the size(ϕ) of the nucleus of a normal hepatocyte($>3.5\,\mu$) were counted by 4.6 ± 3.0, those which were medium size (ϕ, $2.5-3.5\,\mu$) counted by 4.3 ± 3.2, and those which were smaller size (ϕ, $<2.5\,\mu$) were numbered by 3.58 ± 3.2(Fig. 4).

In the T–2 toxin 4 mg/kg treated groups, the maximum numbers were counted at 5 hrs

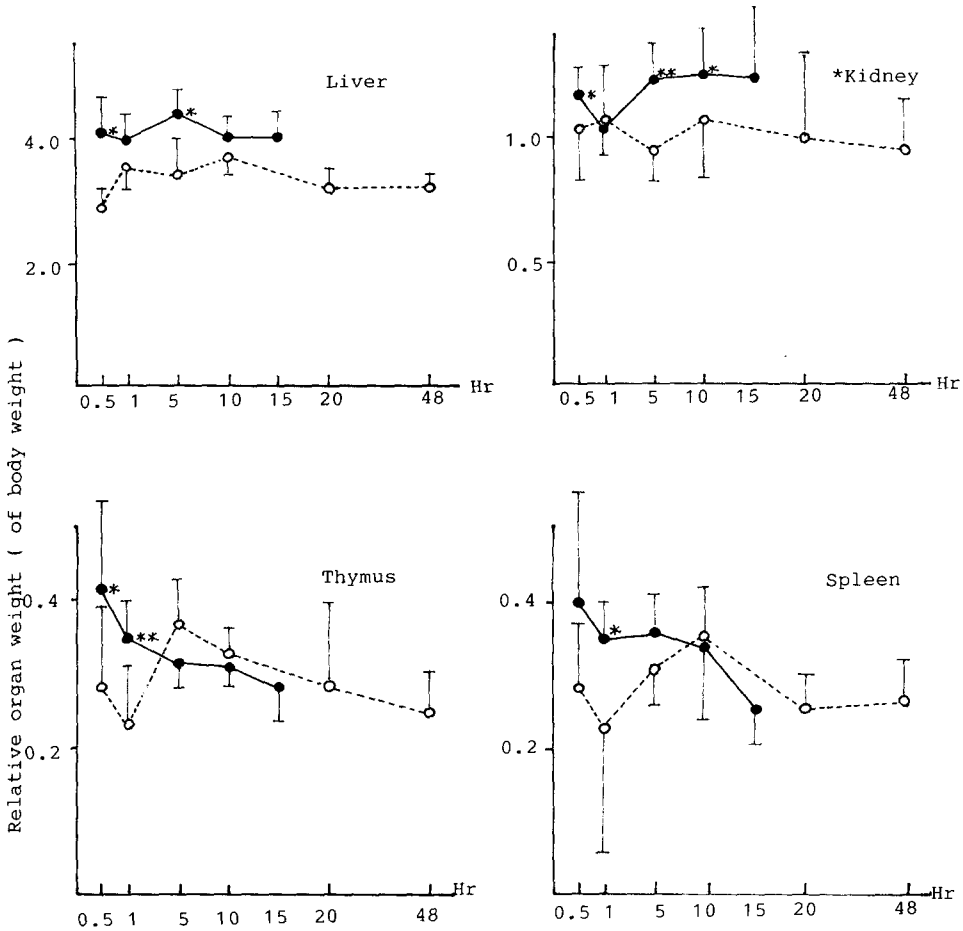


Fig. 3. ○, Control: ●, T-2 toxin. *Sum of right and left kidneys. Singificantly different from control:*P< 0.01. Changes in organ weight in rats administered T-2 toxin. Animals were treated p.o. 4mg of T-2 toxin/kg body weight dissolved in 6.2% EtOH or the vehicle alone. Each value i the figures represents the mean±SD.

and large acidophil bodies were counted by 18.1±10.0, medium sizes were counted by 23.7±12.2, and small acidophil bodies were counted by 49.1±20.4(Fig. 5). It was dose-dependent trends for the frequency of acidophil bodies to show between the 4 mg/kg treated group and the 2 mg/kg treated group. The relative percentage of both maximum numbers are as follows; 402% in large size, 551% in medium size, and 1370% in small size.

The location of acidophil bodies in hepatic lobules revealed even distribution in pericentrilobular, midzonal, and periportal regions. In stainability of eosin, the colors of acidophil bodies appeared clear scarlet, red, and dull hued scarlet. They are also observed isolated forms or colony like forms in view of their formation(Fi.g 6).

In the same preparation, there were slight necrotic coagulative foci in surrounding areas of colony-like formed acidophil bodies. Changed nucleus and its disappearance were also observed in the periphery of acidophil bodies and could not show any damage of the hepato biliary system. Microscopically, irregular basophilic masses appeared by 5 hrs after

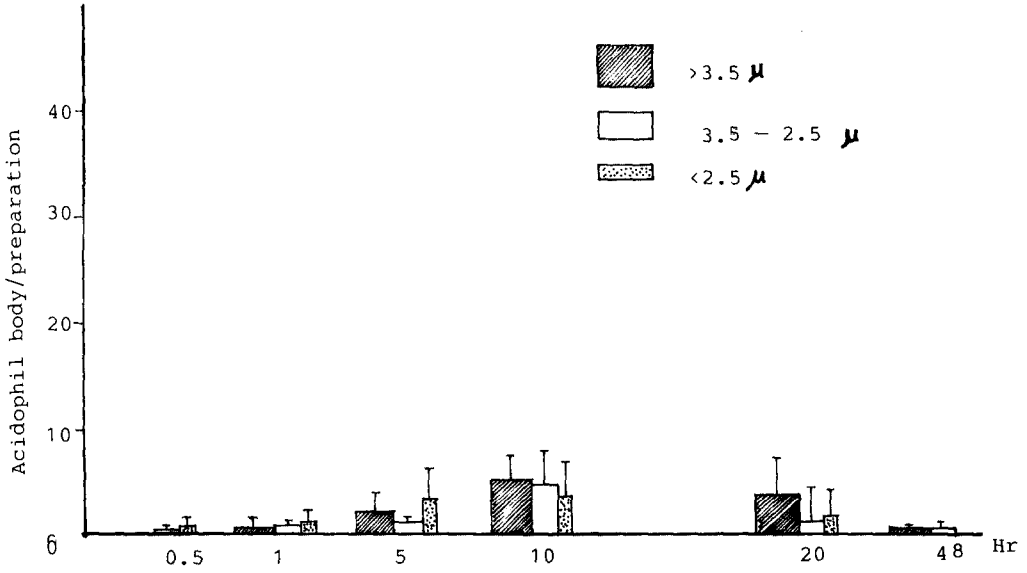


Fig. 4. The histogram of size and count of acidophil body in T-2 toxin 2mg/kg body weight treated group.

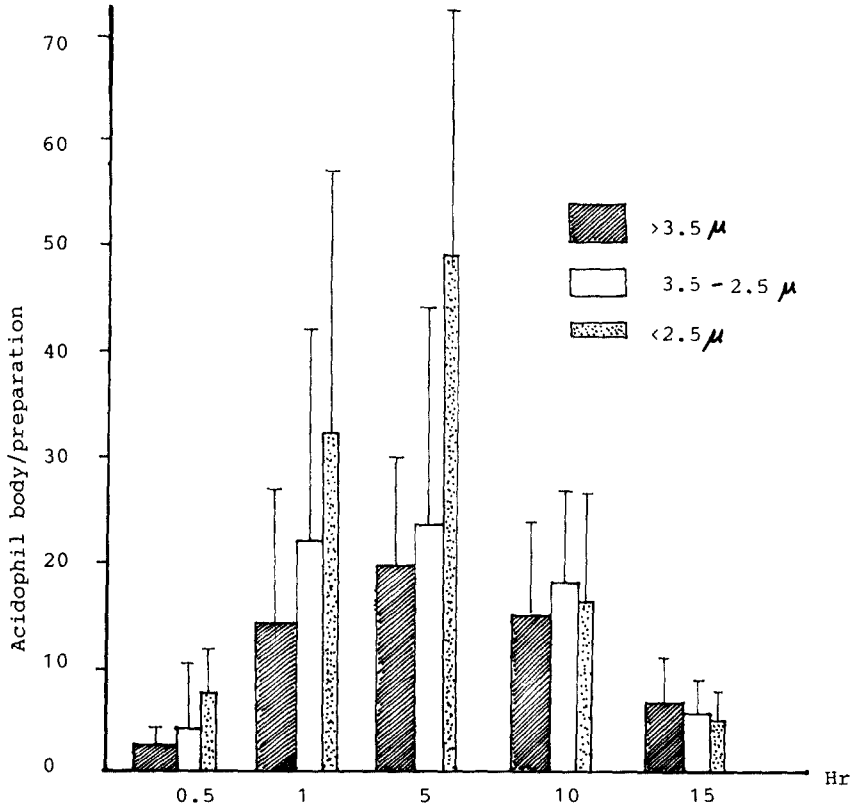


Fig. 5. The histogram of size and count of acidophil body in T-2 toxin 4mg/kg body weight treated group.

dosing(Fig. 11). The basophilic masses have been accepted as corresponding to arrays of cisternae of the RER(Fig. 12).

Overally or selectively located round electron–dense materials and myelinlike changes were observed in mitochondria of hepatocyte 0.5 hr after dosing(Fig. 7).

Electron microscopically, surroundings of some acidophil bodies had shrunken nucleus (Fig. 8). There were electron–dense 2–6 compartments, which consisted of vacuole like inclusions, or fragmented RERs and other organelles, which were barely recognizable(Fig. 8). Fragmented RERs were also observed near the acidophil bodies. Especially, isolated–form acidophil bodies consisted of autophagous vacuoles with double (lysosomal) membranes, glycogen granules and vacuole–like inclusions, several of which contain myelinlike formations(Fig. 10). There were no damage of microvilli in bile canaliculi after dosing (Fig. 13).

DISCUSSION

Administration of a single p.o. dose of T–2 toxin 4 mg/kg produced transient luekocytopenia at 1 hr and decreased 15 hrs after dosing. Chan et al. (1984) reported that intramuscular in the administration of T–2 toxin to rats and rabbits, PCV decreased significantly until 48 hours after dosing. It was due to supression of hematopoietic tissue. WBC was increased transiently 1 and 5 hrs after dosing, and showed an abrupt decrease 15 hrs after dosing. The findings of this research are very similar to previous reports (Kosuri 1971, Sato et al. 1975, Tsuchida et al. 1983).

The value of total protein was decreased 10 and 15 hours after dosig. Since the T–2 toxin usually has been known to cause acute hemorrhages in G 1 tracts by te 'radiomimetic effect' (Sato and Ueno 1977) or 'complex multiple syndrome' (Yarom 1983), the manifestation of diathesis of blood from mucous membrane and feed refusal would be correlated hematological data (Fig. 1). It was interesting point that complete receptor occupation of membranes probably was damaged primarily in addition to the radiomimetic effects (Gyongyossy *et al.* 1985).

Concerning the parenteral administration of T–2 toxin, Lucas et al. (1982) reported that T–2 toxin evoked massive hemorrhages and damage of actively dividing cells. In addition causes of hemorrhages were damage of vascular (Schoental 1979) or direct damage of platelets (Yarom *et al.* 1983) as has been suggested.

There were reports (rat; Tsuchida et al. 1983, cattle; Kosuri 1970) about increasing of the GPT and GOT activities. In reports of ALP activities about T–2 toxicosis in rabbits with IV routes (Gentry and Cooper 1981), in cattle with p.o.(Weaver *et al.* 1980), and in chickens with p.o. (Pearson 1978), decreases of activities reported decreased. Though decreases were observed in rabbits, not any decrease was observed in ALP on rats by Chan *et al.* (1984).

In these studies, overall increases of GPT activities indicated the massive damage of the liver in addition to the permeability changes of hepatocytes, and it was correlated with the frequency of acidophil body in a time course study (Fig. 2,5).

Increases of GPT activities (Fig. 2) and electron–microscopical changes (Fig. 7) indicate the probable damage of mitochondria in hepatocytes, even though GPT activities has been known, more specific in cytoplasmic changes of muscle cells than that of hepatocytes (Maxine 1961).

Some investigators have pointed out the possibility that biomembranes such as the ER and mitochondria (Mead 1976), showing early inhibition as suspected by Pace *et al.* (1983), might be produced through the disorder in the electron transport system accompanied by

membrane structural alteration.

As we found the morphological changes of mitochondria only 0.5 hrs after dosing, these changes probably transient or selective. In our assumption, these changed mitochondria were captured by lysosomal membranes of hepatocytes, as autophagous vacuoles and finally formed to acidophil body.

Suneja *et al.*(1983) reported the increase of liver weights by lipid accumulation in the T–2 toxicosis of rats. The relative weights of livers and kidneys could be elucidated their metabolic pathway, as detoxification of the T–2 toxin, its metabolites, and their excretion (Pace 1985).

Changes of body temperature were similar to the studies of repeated treatment of T–2 toxin p.o. (Kosuri 1970). Recently, Chan et al. (1984) also reported that body temperature of rabbits with the treatment of I.M. injection of T–2 toxin did not show any changes.

In our microscopical observation, we found the randomly spaced slight necrotic foci (Fig. 6) in comparison to the reports of Schoental(1979), Rukmni(1980). In our observation of acidophil bodies (Fig. 4, 5), the frequency of acidophil bodies showed dose–dependent trends. The appearance of acidophil bodies accelerated rapidly in the higher dosed subjects than in the half(2mg/kg) dosed treated group. In addition, they were correlated with the results of GPT activities(Fig. 2) We considered that the healing of the liver process corresponded with very rapid metabolism and excretion of T–2 toxin.

Minute vaculoes in the periphery of hepatocytes were not found in our experiment, so thus differed from the report of Tsuchida et al. (1983), and it was reported also the observation of many hyaline droplets. We would like to suggest the common name 'acidophil body', because hyaline body usually consists of fibril or filament types such as alcoholic hyaline bodies or inclusion in ultrastructure. In acute viral hepatitis, there were two types of cellular degeneration, one is acidophil type, the other is ballooning types(Schaff 1966, Biava *et al.* 1965). In our opinion, morphological characteristics of acidophil bodies are hard to differentiate with light microscopy, namely they look alike by several different causative agents. In our assumption, there are considerable correlations in the evaluation of acute hepatotoxicity tests between T–2 toxin and other hepatotoxic causative agents.

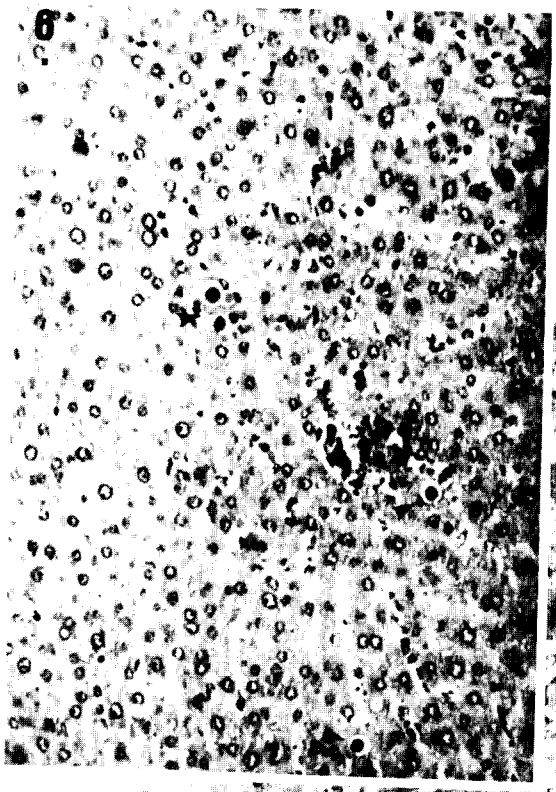
Irregular basophilic masses by 5 hrs (Fig. 11) was very similar in appearance of clumping and fading, which has been reported by Tsuchida *et al.* (1983). We considered that kind of masses would be stacks of 15–17 cisternae, rough endoplasmic reticulum with long band-shapes.

In this present paper, hepatotoxicity of T–2 toxin was not selective, because acidophil bodies had not been always located in a confined specific area of parenchyma cells. Also they probably originated from the cytoplasm of hepatocytes, so the T–2 toxin primarily disordered the cytoplasm rather than the nucleus.

Ultrastructural studies of the observation of filament types (Yokoo et al. 1972, French *et al.* 1975) revealed shrunken nucleus with councilman–like bodies, which did not contain glycogens or ribosmes (Biava *et al.* 1965). But we found that autophagous vacuoles contained glycogen granules and vacuole–like inclusions, several of which myelin like formations.

It is an interesting point that shrunken nucleus(Biava *et al.* 1965) and band–shaped arrangements by aflatoxin B. (Bannasch *et al.* 1985) are similar to our observation by the T–2 toxin.

Electron microscopically, microvilli of bile canaliculi were intact 5hrs after dosing. As in the previous report(Chan *et al.* 1984), we also could not found any decrease of ALP activities-(Fig. 2). Probably, this results is due to the difference of the anatomical structure of the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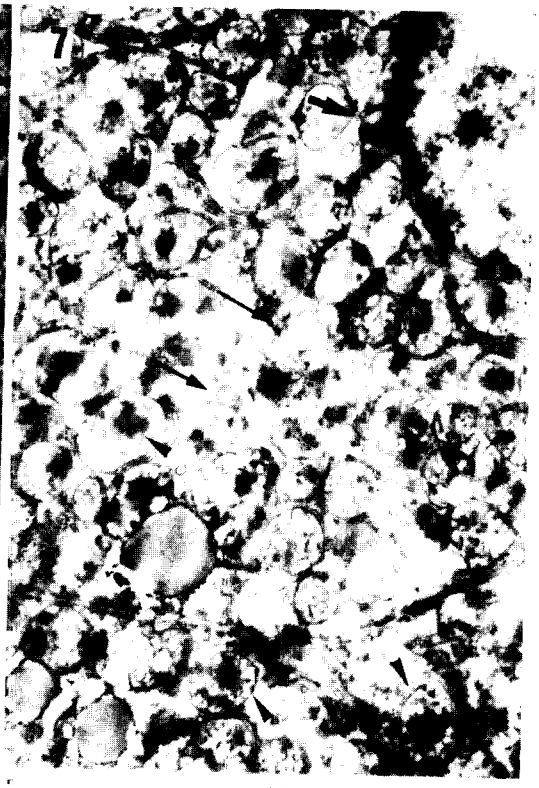
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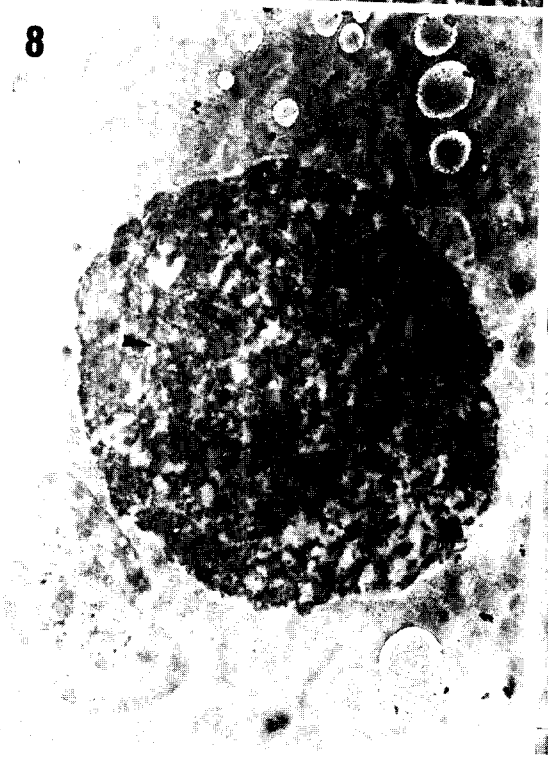
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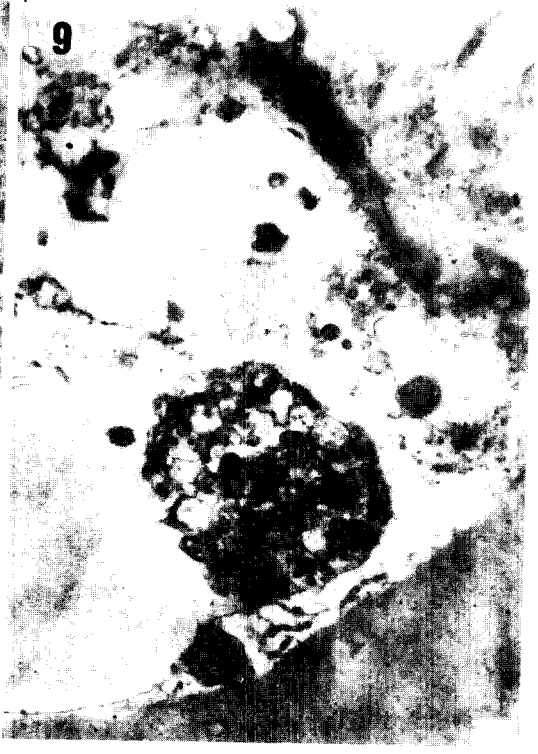
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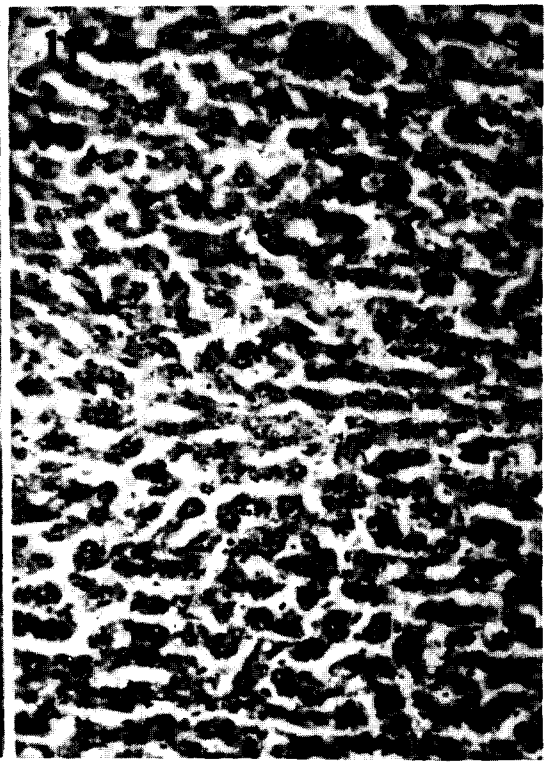

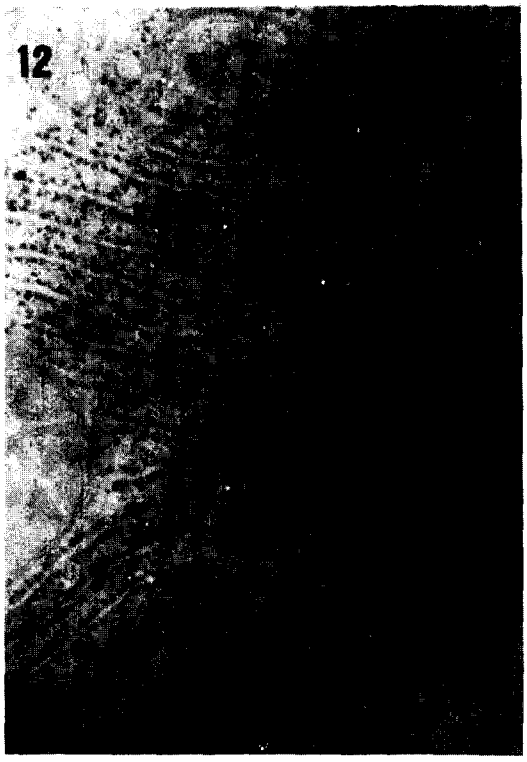
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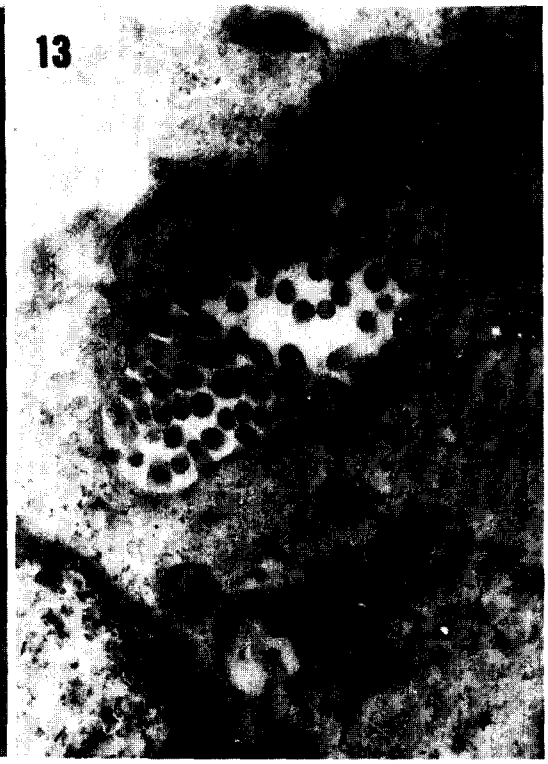
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LEGENDS FOR FIGURES

Fig. 6. Photomicrograph of the liver from a rat at 5 hours after a single p.o. dose of T–2 toxin (4mg/kg of body weight) was given. Note variable size of acidophil bodies. H & E staining; x. 100

Fig. 7. Electron micrograph of the liver of a rat at 0.5 hours after a single p.o dose of T–2 toxin (4mg/kg) was given. Note electron–dense matrix and myelin–like changes of mitochondria. Uranyl acetate–lead citrate staining; x. 6,000.

Fig. 8. Electron micrograph of the liver at 5 hour after dosing. Note shrunken nucleus and fragmented RERs in acidophil body, uranyl acetate–lead citrate staining; x. 6,000.

Fig. 9. Electron micrograph of the liver from a rat 5 hours after a single p.o. dose of T–2 toxin was given. Note acidophil body with vacuole like formations and RERs. uranyl acetate–lead citrate staining; x. 7,000

Fig. 10. Electron micrograph of the liver from a rat 5 hours after a single p.o. dose of T–2 toxin was given. Note pear–shape matrix of mitochondria and acidophil body consist of vacuole like inclusion and myelin like changes of mitochondria. uranyl acetate–lead citrate staining; x. 15,000

Fig. 11. Methylene blue–eosin stain of a rat 1 hour after a single p.o. dose of T–2 toxin was given. Note many basophilic masses in the hepatocytes; x. 400

Fig. 12. Electron micrograph of the liver from a rat 5 hours after a single p.o. dose of T–2 toxin was given. Note RERs with long band–shape arrangements. uranyl acetate–lead citrate staining; x. 12,000.

Fig. 13. Electron micrograph of the liver from a rat 5 hours after a single p.o. dose of T–2 toxin was given. Microvilli of the bile canaliculi are intact. uranyl acetate–lead citrate staining; x. 22,000.

hepatobiliary system in rats. T–2 toxin has been known to exhibit a strong affinity for biomembrane (Iwahashi *et al* 1982). Lipid peroxidation has been taken as an important factor causing membrane injury (Mead 1976). Therefore, the elucidation of the real mechanism is still in progress. So, the following pathogenesis of T–2 toxin, which inlcuded acidophil body, is strongly needed not only ultrastructural studies but also various animal testing of experimental animals.

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