



T-2 toxin-induced acute skin lesions in Wistar-derived hypotrichotic WBN/ILA-Ht rats

S.M. Albarenque, J. Shinozuka, S. Iwamoto, H. Nakayama and K. Doi.

Department of Veterinary Pathology, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

Summary. Acute lesions in the dorsal skin topically applied with T-2 toxin (10 μ l of 0.5 mg/ml-solution to 1cm²) were examined in Wistar-derived hypotrichotic WBN/ILA-Ht rats up to 24 hours after treatment (24HAT). In the epidermis, depression of basal cell proliferating activity was detected at 3HAT by immunostaining for proliferating cell nuclear antigen (PCNA), and the percentage of PCNA-positive basal cells decreased thereafter. At 12HAT, in addition to intracytoplasmic edema of spinous cells, acidophilic degeneration of basal cells characterized by shrinkage of cell body with acidophilic cytoplasm and pyknotic or karyorrhectic nuclei became prominent. Most of these nuclei were positive for TUNEL which is a widely used immunostaining for the *in situ* detection of fragmented DNA, i.e. apoptosis, and the percentage of TUNEL-positive basal cells increased thereafter. The nuclei of these basal cells also showed ultrastructural changes characteristic for apoptosis. On the other hand, in the dermis, infiltration of inflammatory cells including mast cells started at 3HAT and increased thereafter. In addition, capillary and small vessel endothelial degeneration developed at 6HAT and progressed thereafter. These results suggest that T-2 toxin directly affects the epidermis and produces apoptosis in basal cells.

Key words: Apoptosis, Basal cell, Skin lesion, T-2 toxin, WBN/ILA-Ht rat

Introduction

T-2 toxin is a kind of trichothecene mycotoxins produced by the genus *Fusarium* (Ueno 1980; IARC, 1993), and has been implicated in several diseases in grain handling workers as well as in domestic animals usually by oral exposure. T-2 toxin can also penetrate through the skin (Kemppainen et al., 1984; Bunner et al.,

1989; Solberg et al., 1990), and some researchers reported the local (Yarom et al., 1987; Bhavanishankar et al., 1988) as well as generalized injury (Shiefer and Hancock, 1984; Blaylock et al., 1993) caused by topical application of T-2 toxin on the dorsal skin. This seems to be important because there is a chance that the skin of grain handling workers as well as of domestic animals is exposed to T-2 toxin-contaminated grain dust.

It is generally said that T-2 toxin produces lesions in hematopoietic (DeLoach et al., 1989; Smith et al., 1994), lymphoid (Rotter et al., 1994) and gastrointestinal tissues (Lutsky and Mor, 1981; William, 1989) which contain many actively proliferating cells, resulting in lymphopenia, anemia and diarrhea. We recently clarified that it is brought about by apoptotic cell death in hematopoietic (Shinozuka et al., 1998) and lymphoid cells (Quiroga et al., 1993; Li et al., 1997; Shinozuka et al., 1997a,b) as well as in intestinal crypt epithelial cells (Li et al., 1998).

The aim of this study is to clarify the details of T-2 toxin-induced acute skin lesions using Wistar-derived hypotrichotic WBN/ILA-Ht rats (HtRs). HtR has an autosomal dominant gene (*Ht*: dominant hypotrichosis) responsible for the characteristics of hypotrichosis and is considered to be a useful laboratory animal for dermatotoxicity study (Iwamoto et al., 1997, 1998).

Materials and methods

Animals

Five 7-week-old male WBN/ILA-Ht rats (Saitama Experimental Animals Co., Saitama) were used. They were kept in an animal room using isolator caging system (Niki Shoji Co., Tokyo) under controlled conditions (temperature: 23 \pm 2 °C; relative humidity: 55 \pm 5%) and fed standard diet (MF, Oriental Yeast Co. Ltd., Tokyo) and water *ad libitum*.

Chemicals

T-2 toxin (Lot. No. 117F4078, Sigma Co., St. Louis, MO) dissolved in 20% ethanol was used.

Offprint requests to: Dr. Stella M. Albarenque, Department of Veterinary Pathology, Faculty of Agriculture, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

Treatments and sampling

Ten μl of T-2 toxin solution (0.5 mg/ml) was topically applied to 4 areas (1 cm^2 each) of the dorsal skin of each animal, and 10 μl of solvent alone to 2 areas, respectively. The dose of T-2 toxin was determined based on the results of preliminary dose-finding study. Tissue specimens were obtained from the T-2 toxin-treated sites of 5 animals with a 6 mm biopsy punch (Nagatoishi Co. Ltd., Tokyo) under ether anesthesia at 3, 6, 12, and 24 hours after treatment (HAT), respectively. In addition, tissue specimens were also obtained from the solvent-treated sites of 5 animals at 0 and 24 HAT, respectively.

Histopathology

Skin specimens were fixed in 10% neutral-buffered formalin, and 4- μm paraffin sections were stained with hematoxylin and eosin (HE) and toluidine blue (TB) for histopathological examination. The number of mast cells in the dermis was counted on TB-stained section under light microscope and mean \pm standard deviation (SD) / mm^2 of 5 animals was calculated at 3, 6, 12 and 24 HAT for the T-2 toxin-treated skin and at 0 and 24 HAT for the solvent-treated skin, respectively.

In situ detection of fragmented DNA

DNA fragmentation was examined on the paraffin sections by the modified TUNEL method first proposed by Gavrieli et al. (1992), using a commercial apoptosis detection kit (ApopTag, Oncor, Gaithersburg, MD). In brief, the procedure was as follows: multiple fragmented DNA 3'-OH ends on the paraffin sections were labeled with digoxigenin-dUTP in the presence of terminal deoxynucleotidyl transferase (TdT). Peroxidase-

conjugated anti-digoxigenin antibody was then reacted with the sections. Apoptotic nuclei were visualized by peroxidase-diaminobenzidine (DAB) reaction. The sections were then counterstained with methylgreen. The ratio of TUNEL-positive cells to all the epidermal basal cells was calculated on one section/animal under light microscope, and then mean \pm SD of 5 animals was expressed as % at 3, 6, 12 and 24 HAT for the T-2 toxin-treated skin and at 0 and 24 HAT for the solvent-treated skin, respectively.

Immunohistochemical staining for PCNA

For evaluating the proliferative activity of epidermal basal cells, immunohistochemical staining for proliferating cell nuclear antigen (PCNA) was carried out on paraffin sections by the avidin-biotin-peroxidase complex method using ABC kit (Vector Lab. Inc., Novocastra, Newcastle, UK). Mouse anti-rat PCNA antibody (clone PC10; Novocastra, Newcastle, UK) was used as the primary antibody. The sections were visualized by DAB reaction and then counterstained with methylgreen. The ratio of PCNA-positive cells to all the epidermal basal cells was calculated on one section/animal under light microscope, and the mean \pm SD of 5 animals was expressed as % at 3, 6, 12 and 24 HAT for the T-2 toxin-treated skin and at 0 and 24 HAT for the solvent-treated skin, respectively.

Electron microscope

For electron microscopic examination, small pieces of the skin samples were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4), postfixed in 1% osmium tetroxide in the same buffer, and embedded in EPOK 812 (Ohken Co. Ltd., Tokyo). Ultrathin sections were double stained with uranyl acetate and lead citrate

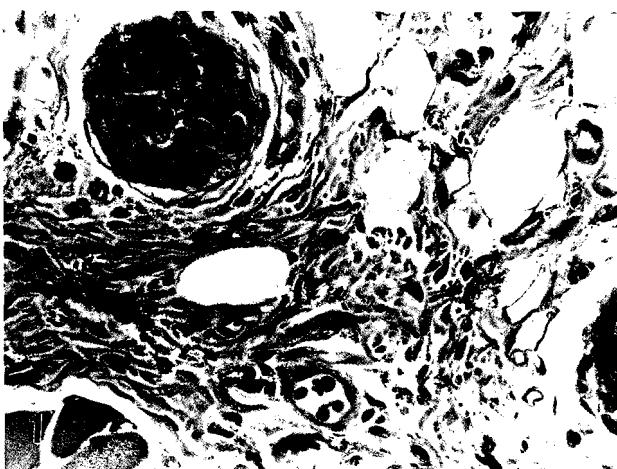


Fig. 1. T-2 toxin-treated skin of an HtR at 3HAT. Mild infiltration of inflammatory cells including mast cells is seen in the deeper dermis. HE. x 280

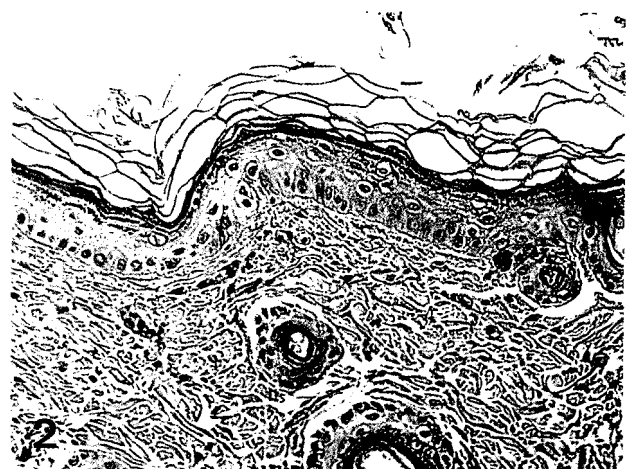


Fig. 2. T-2 toxin-treated skin of a HtR at 3HAT. No histopathological changes are seen in the epidermis. HE. x 90

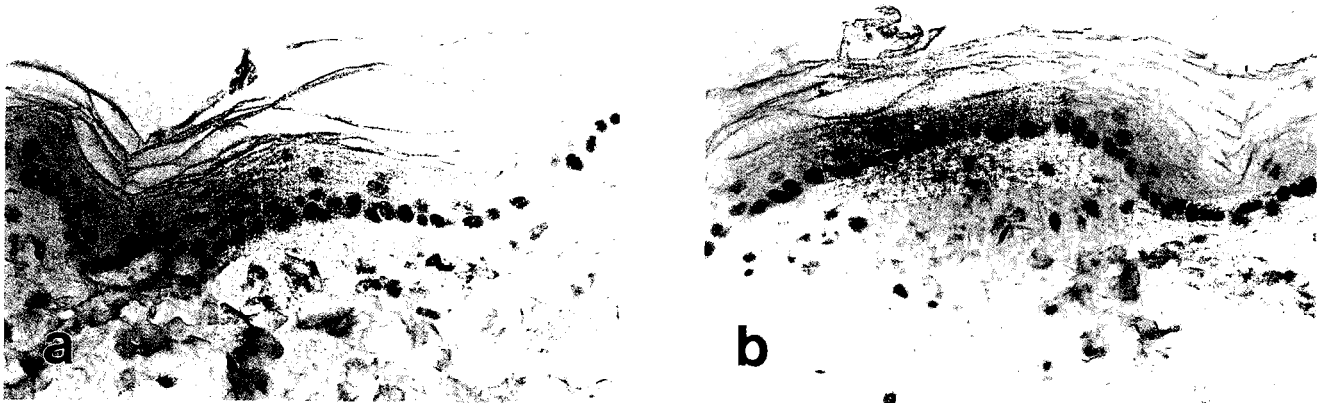


Fig. 3. Solvent-treated skin of an HtR at 0 HAT (a) and T-2 toxin-treated skin of an HtR at 3HAT (b). The number of PCNA-positive basal cells is less in (b) than in (a). Immunostaining. x 320

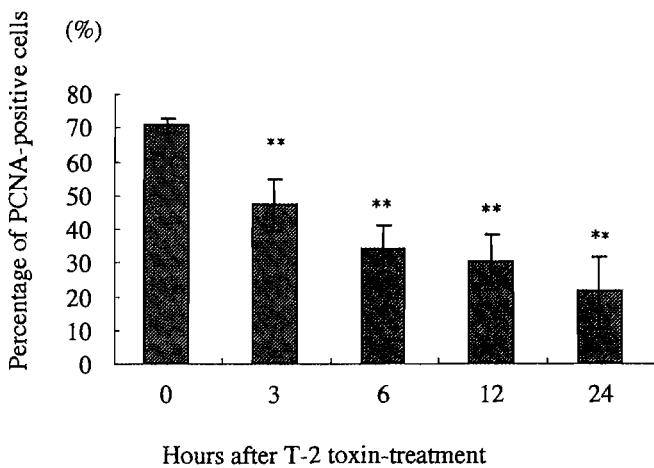


Fig. 4. Changes in percentage of PCNA-positive cells (mean \pm SD of 5 animals) in the basal cell layer of the epidermis of HtRs after topical application of T-2 toxin. **: $p < 0.01$ (statistically significant from control).

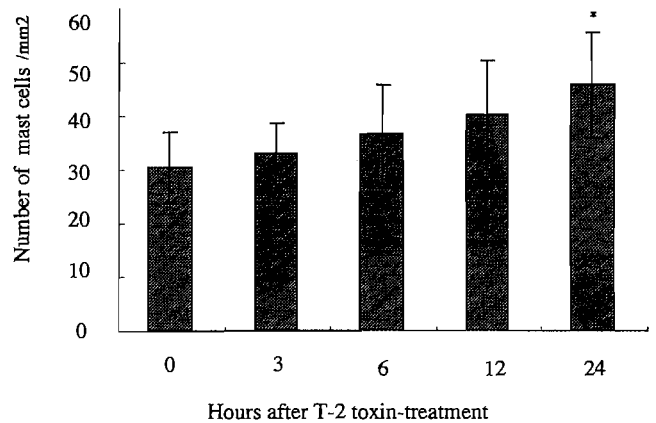


Fig. 5. Changes in number of mast cells (mean \pm SD of 5 animals) in the dermis of HtRs after topical application of T-2 toxin. *: $p < 0.05$ (statistically significant from control).



Fig. 6. T-2 toxin-treated skin of an HtR at 12HAT. Intracytoplasmic edema of spinous cells and acidophilic degeneration of basal cells are seen in the epidermis. HE. x 550

and observed under a JEM-1200 EX electron microscope (JEOL Co. Ltd., Tokyo).

Statistical analysis

Statistical analysis was done on the number of mast cells and the percentages of TUNEL-positive and PCNA-positive cells using Student's t-test.

Results

In the T-2 toxin-treated skin, mild neutrophilic infiltration with slight increase in number of mast cells was observed in the deeper dermis and subcutis at 3 HAT (Fig. 1). At this time, although the epidermis showed no histopathological changes (Fig. 2), the number of PCNA-positive epidermal basal cells significantly decreased (Fig. 3). Thereafter it continued to decrease until 24 HAT (Fig. 4). The number of mast cells tended to increase with time especially around the

T-2 Toxin-induced skin lesions in rats

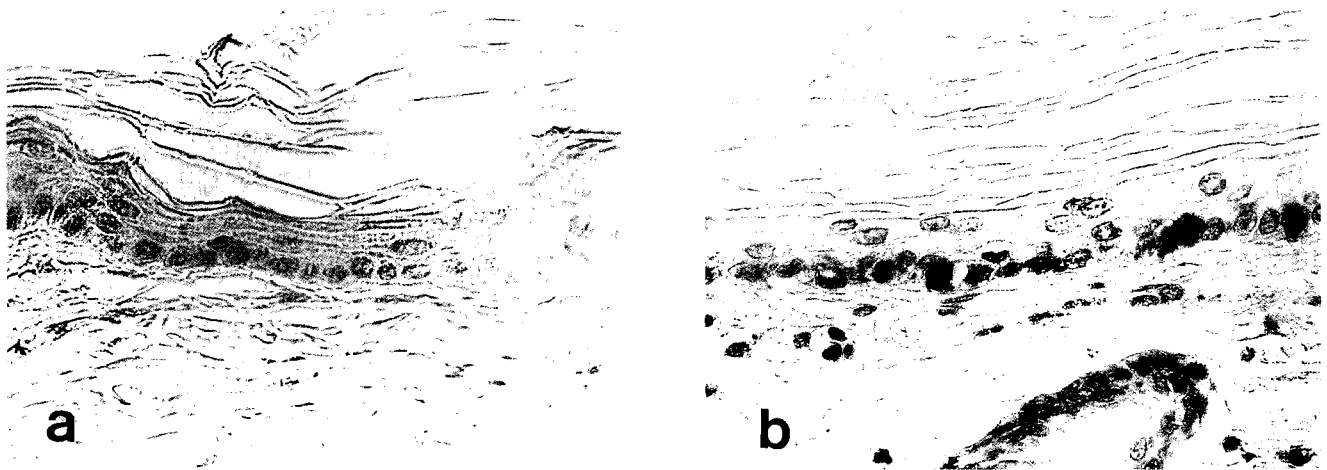


Fig. 7. Solvent-treated skin of an HtR at 0 HAT (a) and T-2 toxin-treated skin of an HtR at 12HAT (b). TUNEL-positive cells are seen in the basal cell layer of the epidermis of (b). Immunostaining. x 450

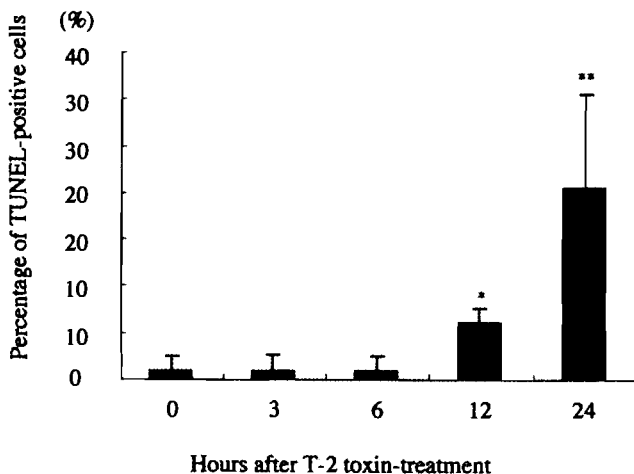


Fig. 8. Changes in percentage of TUNEL-positive cells (mean \pm SD of 5 animals) in the basal cell layer of the epidermis of HtRs after topical application of T-2 toxin. *: $p < 0.05$, and **: $p < 0.01$ (statistically significant from control).

small vessels in the dermis (Fig. 5), but degranulation of mast cells was not evident. Inflammatory cell infiltration also increased with time.

At 12 HAT, intracytoplasmic edema of spinous cells and acidophilic degeneration of basal cells developed in the epidermis. Basal cells showing acidophilic degeneration were characterized by shrinkage of cell body with acidophilic cytoplasm and pyknotic or karyorrhectic nuclei (Fig. 6). These nuclei were generally TUNEL-positive (Fig. 7), and the number of TUNEL-positive epidermal cells significantly increased at 12 and 24 HAT (Fig. 8).

Electron microscopically, basal cells characterized by acidophilic degeneration showed condensation of nuclear chromatin or margination of condensed

chromatin along the nuclear membrane (Fig. 9), and the nuclei of some basal cells were fragmented into small pieces. In the dermis, mild degenerative changes were sporadically observed in the capillary and small vessel endothelial cells at and after 6HAT (Fig. 10).

At 24 HAT, degenerative changes in the epidermis progressed more (Fig. 11). In the dermis and subcutis, in addition to inflammatory cell infiltration, such changes as dilatation, stasis and/or endothelial cell degeneration developed in many capillaries and small vessels, resulting in edema (Fig. 12).

On the other hand, in the solvent-treated skin, there were no abnormal histopathological findings. In addition, there were no differences in the number of mast cells, PCNA-positive basal cells and TUNEL-positive basal cells between skin samples obtained at 0 and 24 HAT.

Discussion

Details of acute skin lesions induced in the dorsal skin of HtRs by topical application of T-2 toxin ($10 \mu\text{l}$ of 0.5 mg/ml solution in 1 cm^2) were examined. Previous reports of T-2 toxin-induced skin lesions suggested that the microvessels in the dermis and subcutis were initially damaged and that the epidermal changes were secondary to ischemia brought about by the above-mentioned microvessel damage (Yarom et al., 1987). On the contrary, in the present study, degenerative changes were detected at the same time or somewhat later in the vessels than in the epidermis even in electron microscopic examinations, and this suggests the direct toxic effect of T-2 toxin on the epidermis. Yarom et al. (1987) also reported that T-2 toxin directly damaged mast cells in the dermis, resulting in degeneration of mast cells. In the present study, except for mild increase in number of mast cells with time, no apparent morphological changes were detected in mast cells.

From the results of immunohistochemical staining for PCNA, prior to the development of histopathological

T-2 Toxin-induced skin lesions in rats

changes in the epidermis, significant depression of cell proliferating activity occurred at 3 HAT in the basal cell layer; which contains cells with high mitotic activity. This indicates that topically applied T-2 toxin directly affects the epidermis by its radiomimetic activity. At 12 HAT, prominent degenerative changes developed in the epidermis, and intracytoplasmic edema was characteristic for spinous cells and acidophilic degeneration for basal cells, respectively. The cause of the difference in the histopathological characteristics between spinous cells and basal cells is obscure. Basal cells showing

acidophilic degeneration were characterized by shrinkage of cell body with acidophilic cytoplasm and pyknotic or karyorrhectic nuclei. These nuclei were generally TUNEL-positive, and showed ultrastructural characteristics of apoptosis such as condensation of nuclear chromatin, margination of condensed chromatin along the nuclear membrane or fragmentation. These histopathological, TUNEL-staining and electron microscopic findings were similar to those of apoptosis in the hematopoietic (Shinozuka et al., 1998), lymphoid (Li et al., 1997; Shinozuka et al., 1997a,b) and intestinal tissues (Li et al., 1998) previously reported by us.

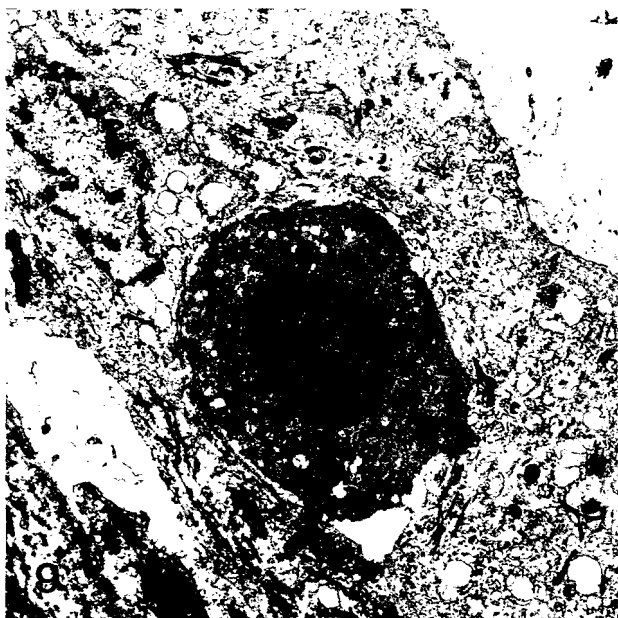


Fig. 9. Basal cell of T-2 toxin-treated skin of an HtR at 12HAT. Margination of condensed chromatin along the nuclear membrane. x 6,000

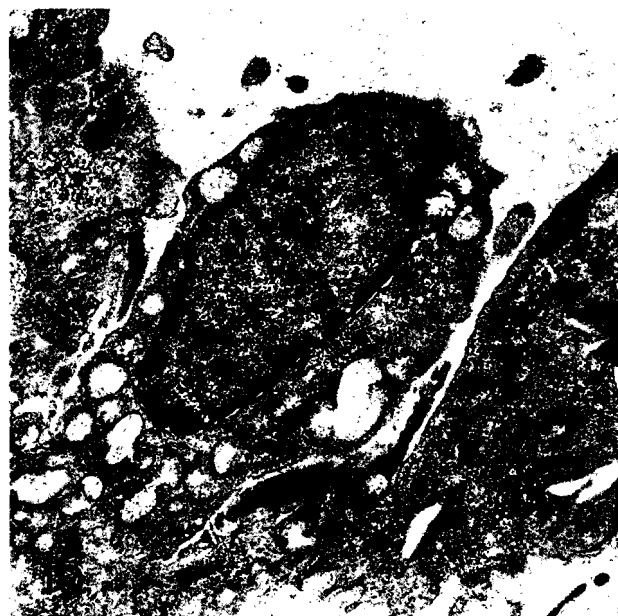


Fig. 10. Capillary endothelium of T-2 toxin-treated skin of an HtR at 6HAT. Degeneration of endothelial cell. x 8,000

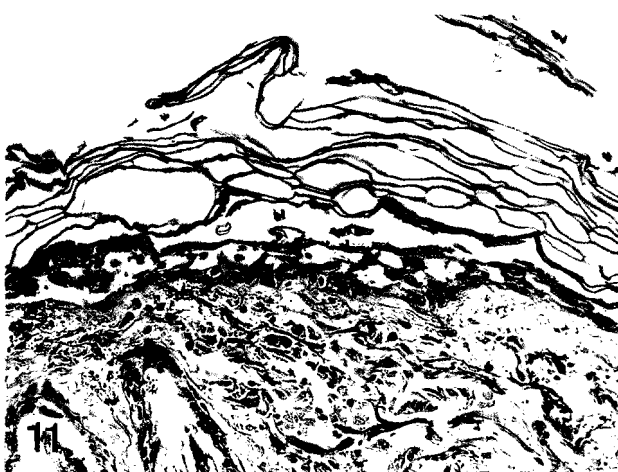


Fig. 11. T-2 toxin-treated skin of an HtR at 24HAT. Marked degenerative changes are seen not only in the epidermis but also in the dermis. HE. x 100

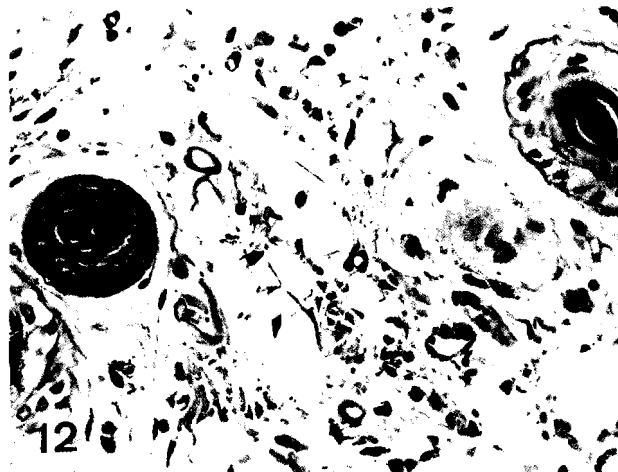


Fig. 12. T-2 toxin-treated skin of an HtR at 24HAT. Capillary endothelial degeneration with inflammatory cell infiltration and edema is seen. HE. x 240

T-2 Toxin-induced skin lesions in rats

Shiefer and Hancock (1984) reported that topical application of 5-40 ng/kg b.w. of T-2 toxin to the dorsal skin of mice induced lesions in lymphoid, hematopoietic and intestinal tissues. However we, could not detect such lesions (data not shown) in the present study.

In conclusion, T-2 toxin topically applied to the dorsal skin of WBN/ILA-*Ht* rats brought about significant depression of cell proliferating activity in the basal cell layer of epidermis as the earliest apparent effect, and apoptosis developed in the same layer of the epidermis several hours later.

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