

20030130136

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AD-A198 050 DOCUMENTATION PAGE

Form Approved  
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified			1b. RESTRICTIONS DTIC FULL		
2a. SECURITY CLASSIFICATION AUTHORITY DTIC ELECTE			3. DISTRIBUTION AVAILABILITY OF REPORT Distribution unlimited - approved for public release		
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE AUG 15 1988			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
4. PERFORMING ORGANIZATION REPORT NUMBER(S)			7a. NAME OF MONITORING ORGANIZATION U.S. Army Medical Research and Development Command		
6a. NAME OF PERFORMING ORGANIZATION U.S. Army Medical Research Institute of Infectious Diseases		6b. OFFICE SYMBOL (if applicable) SGRD-UIP-C	7b. ADDRESS (City, State, and ZIP Code) Fort Detrick, Frederick, MD 21701-5012		
6c. ADDRESS (City, State, and ZIP Code) Fort Detrick, Frederick, MD 21701-5011			9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER		
8a. NAME OF FUNDING/SPONSORING ORGANIZATION		8b. OFFICE SYMBOL (if applicable)	10. SOURCE OF FUNDING NUMBERS		
8c. ADDRESS (City, State, and ZIP Code)			PROGRAM ELEMENT NO	PROJECT NO	TASK NO
11. TITLE (Include Security Classification) Testosterone prevents T-2 toxin-induced adrenal cortical necrosis in mice					
12. PERSONAL AUTHOR(S) J.D. Thurman, D.A. Grensias, and R.W. Trotter					
13a. TYPE OF REPORT Interim		13b. TIME COVERED FROM TO		14. DATE OF REPORT (Year, Month, Day) 7 April 1988	
15. PAGE COUNT 8					
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP			
19. ABSTRACT (Continue on reverse if necessary and identify by block number) To evaluate the effect of exogenous testosterone on the occurrence of T-2 toxin-induced necrosis of adrenal glands, mice were divided into 3 treatment groups. Group 1 mice received 12 subcutaneous injections of testosterone at 48-hr intervals prior to aerosol exposure to the toxin; group 2 mice received similar injections of only the vehicle, and group 3 mice received no treatment. Each treatment group contained castrated male, castrated female, and intact female mice. All mice alive 24-hr after a 10-min exposure to T-2 toxin aerosol were killed and the adrenal glands and thymuses examined histologically. Necrosis of the adrenal cortex was not present in any of the mice receiving preexposure treatment with exogenous testosterone (group 1). All mice receiving vehicle only (group 2) or no treatment (group 3) had T-2 toxin-induced necrosis of the inner adrenal cortex. Additionally, the presence of lymphocytolysis in the cortex of the thymus, confirmed that each mouse of all 3 treatment groups had evidence of systemic					
20. DISTRIBUTION AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION		
22a. NAME OF RESPONSIBLE INDIVIDUAL			22b. TELEPHONE (Include Area Code)		22c. OFFICE SYMBOL

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DTIC TAB	<input type="checkbox"/>
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Testosterone prevents T-2 toxin-induced adrenal cortical necrosis in mice

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Key words: T-2 Mycotoxin, adrenal gland, testosterone, mice

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care. The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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## Testosterone prevents T-2 toxin-induced adrenal cortical necrosis in mice

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### SUMMARY

To evaluate the effect of exogenous testosterone on the occurrence of T-2 toxin-induced necrosis of adrenal glands, mice were divided into 3 treatment groups. Group 1 mice received 12 subcutaneous injections of testosterone at 48-hr intervals prior to aerosol exposure to the toxin; group 2 mice received similar injections of only the vehicle, and group 3 mice received no treatment. Each treatment group contained castrated male, castrated female, and intact female mice. All mice alive 24-hr after a 10-min exposure to T-2 toxin aerosol were killed and the adrenal glands and thymuses examined histologically. Necrosis of the adrenal cortex was not present in any of the mice receiving preexposure treatment with exogenous testosterone (group 1). All mice receiving vehicle only (group 2) or no treatment (group 3) had T-2 toxin-induced necrosis of the inner adrenal cortex. Additionally, the presence of lymphocytolysis in the cortex of the thymus, confirmed that each mouse of all 3 treatment groups had evidence of systemic mycotoxicosis. The consistent severity of the thymic lesion in all mice suggests that the thymic lesion was unaffected by exogenous testosterone administration or the castration status of the mice. We propose that, in mice, T-2 toxin-induced adrenal necrosis is prevented by the presence of testosterone.

T-2 toxin is a trichothecene mycotoxin produced by *Fusarium* fungi and is a potent inhibitor of eukaryotic protein synthesis.<sup>1,2</sup> The mycotoxin is cytotoxic for immature lymphoid, hematopoietic, and intestinal crypt epithelial cells.<sup>3,4</sup> In addition to these commonly observed lesions, histopathological changes have been reported in several organs, including

pancreas and heart.<sup>5,6</sup> We recently reported the sequential development of lesions in mice after exposure to T-2 toxin aerosol.<sup>7</sup> In an additional paper, we also reported that exposure to T-2 toxin aerosol caused adrenal cortical necrosis in female, but not male, mice.<sup>8</sup> In a subsequent unpublished study, we found that the toxin caused similar adrenal cortical necrosis in male mice castrated either before or after puberty.<sup>a</sup>

We therefore hypothesized that, in mice, T-2 toxin-induced adrenal cortical necrosis occurs only in the absence of testosterone. The purpose of the present study was to test this hypothesis by studying the effect of exogenous testosterone on T-2 toxin-induced necrosis in the adrenal gland of castrated male, castrated female, and intact female mice.

#### Materials and Methods

*Animals*—Castrated male, castrated female, and sham-operated (intact) female, CD-1 mice, 6 months of age, were obtained from Charles River Laboratories, Stoneridge, NY. They were grouped 6 per open-bottom polycarbonate cage and fed a commercially prepared ration<sup>b</sup> and water ad libitum. Room conditions were maintained at 24 C and 50% relative humidity; room air was changed 12 times per hour. The light cycle was 12 hr. The mice were acclimated (4 to 6 weeks) before the studies were begun.

*Chemicals*—Purified (>99%) T-2 mycotoxin was obtained as a white crystalline powder.<sup>c</sup> Purity was confirmed in this laboratory to be >99% by both thin-layer chromatography and gas chromatography/mass spectrometry analyses. Synthetic [<sup>3</sup>H] mycotoxin in ethanol was obtained from Amersham International, Amersham, UK (sp act: 11 Ci/mmol; 1.1  $\mu$ mol/ml).

Testosterone propionate was obtained as a white crystalline powder.<sup>d</sup> Sesame oil was used as the injection vehicle.<sup>e</sup> Testosterone was mixed with the vehicle at a concentration of 1.6 mg/ml.

*Testosterone treatment*—Prior to T-2 aerosol exposure, mice were divided into 3 treatment groups: Group 1 was testosterone-treated; group 2

was vehicle control; and group 3 received no treatment. Group 1 consisted of 6 castrated male mice, 6 castrated female mice, and 6 intact female mice. At 48 hr intervals, each mouse in Group 1 received a subcutaneous injection of 0.16 mg testosterone propionate in 0.10 ml sesame oil for a total of 12 injections. Group 2 consisted of 3 castrated male mice, 3 castrated female mice, and 3 intact female mice. These mice received 0.10 ml vehicle only on the same injection schedule as group 1. Group 3 consisted of 3 castrated male mice, 3 castrated female mice, and 3 intact female mice. These mice received no treatment prior to T-2 aerosol exposure.

*Aerosol generation*—Crystalline T-2 mycotoxin was dissolved in pharmaceutical-grade ethanol to produce a final solution of 25 mg/ml. Fifty  $\mu$ l of [ $^3$ H]T-2 mycotoxin in ethanol was added as a tracer. The test aerosol was generated by nebulizing the ethanol solution of T-2 with a Lovelace nebulizer.<sup>f</sup>

*Aerosol exposure*—The dynamically operated aerosol chamber was designed for nose-only exposure to the T-2 mycotoxin aerosol. The 10-min exposures were accomplished by placing each mouse in an open-ended, cone-shaped holder with a hole small enough so that only the nose protruded into a 1.5 L aerosol chamber. The chamber was operated at 2.2 L/min. The mice were exposed in 3 groups containing 12 mice each. The aerosol mass concentration varied between 225 to 275  $\mu$ g of T-2/L of air and was determined for each aerosol exposure from a grab sample taken from the aerosol exposure chamber. Grab samples were obtained by drawing the T-2 aerosol from the exposure chamber across a fiberglass filter<sup>g</sup> at the rate of 1.0 L/min for 1.0 min. The fiberglass filter was then placed in a standard scintillation vial. T-2 was extracted overnight from the filter in 1.0 ml of ethanol, 10 ml of scintillation fluid was added, and the [ $^3$ H] on the filter was quantitated by scintillation counting.<sup>h</sup> The quantity of T-2 on the filter was then calculated based on the known ratio of [ $^3$ H]T-2:T-2 that was

present before nebulization. Mice in all 3 groups were exposed to the aerosol 24 hr after the last injection was given to groups 1 and 2.

*Light microscopy*—Adrenal glands and thymus from each animal surviving 24 hr after aerosol exposure were collected at necropsy and fixed in 10% neutral buffered formalin. After paraffin embedding, sections of each tissue were cut at 5  $\mu$ m and stained with hematoxylin and eosin (H&E). All tissue sections were examined by light microscopy. The extent of necrosis was characterized as minimal, mild, moderate, severe. The criteria for necrosis included any of the following: nuclear pyknosis, karyolysis, karyorrhexis, or lysis of plasma cell membrane. Two veterinary pathologists (JDT, TWT) independently evaluated the 2 target tissues for evidence of necrosis. The final results were obtained with the concurrence of both pathologists.

## Results

Immediately after the aerosol exposure, 3 castrated female mice from the testosterone treatment group died. Additionally, one sham-operated (intact) female mouse from the vehicle-only group and one castrated female mouse from the no treatment group died approximately 15 hr postexposure. These 5 mice were not necropsied.

### Group 1

We did not observe adrenal cortical necrosis in any of the mice treated with testosterone and subsequently exposed to T-2 aerosol. There was necrosis of lymphocytes in the cortex of the thymus in each of the mice in group 1. This lymphocytolysis was a striking lesion involving approximately 60% to 90% of the lymphocytes.

### Group 2

Adrenal cortical necrosis was observed in each of the mice injected with vehicle only before exposure to T-2 aerosol. The adrenal lesion was similar to the one previously reported in female mice.<sup>7,8</sup> Necrosis was

restricted to parenchymal cells in a zonal distribution within the zona fasciculata (fig 1). The innermost cells were always affected while there was some variability in the extent of the necrosis into the outer zona fasciculata. Borders of the necrotic zone were generally well demarcated by an abrupt change from necrotic cells to normal cells. Organization of parenchymal architecture within the necrotic areas usually remained discernible due to structural support from unaffected radiating capillaries. The necrotic zone often had sparse numbers of neutrophils present (fig 2). In addition, we observed necrosis of lymphocytes in the thymic cortex in each of these mice. This lymphocytolysis was similar in severity to that present in mice from group 1.

### Group 3

Adrenal cortical necrosis as well as necrosis of lymphocytes in the thymic cortex was observed in each of the untreated mice exposed to T-2 toxin aerosol. Distribution and severity of the adrenal necrosis were similar to that present in group 2 mice. The percent of lytic lymphocytes was analogous to that observed in mice of both group 1 and group 2.

### Discussion

In previous papers we reported that necrosis of lymphocytes in the thymic cortex was an early and sensitive indicator of T-2 toxicosis in both male and female mice. In the present study, we used necrosis of thymic lymphocytes to confirm the presence of T-2-induced toxicosis in each mouse. Since this lesion was present with similar severity in all mice examined histologically, we confirmed the presence of mycotoxicosis in each mouse. Additionally, this lymphocytolysis in the thymic cortex of all mice examined histologically suggests that occurrence of the thymic lesion is not affected by castration status or by the treatment received prior to exposure to the aerosol.

Our original paper reported that a sublethal dose of T-2 toxin causes adrenal cortical necrosis in female, but not male, mice.<sup>6</sup> In the present

paper we report that a similar sublethal dose of T-2 toxin produced adrenal cortical necrosis in castrated male and castrated female in addition to intact female mice. We also report that testosterone treatment prior to T-2 toxin aerosol exposure prevented adrenal cortical necrosis in each of the above groups (i.e., castrated male, castrated female, and intact female). These data support our original report that adrenal necrosis caused by sublethal doses of T-2 toxin does not occur in intact male mice. We further conclude that the presence or absence of ovarian hormones had no effect on occurrence of the adrenal cortical necrosis, as the necrosis was present in all of the intact (sham-operated) female, castrated female, and castrated male mice that did not receive exogenous testosterone.

Further studies are needed to elicit the mechanism(s) by which testosterone protects adrenal cortical parenchymal cells from the necrotizing effects of T-2 toxin.

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<sup>a</sup> Thurman JD, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD: In Preparation, 1987.

<sup>b</sup> NIH formula 07, Zeigler Brothers Inc, Gardners, Pa.

<sup>c</sup> Myco-Lab Co, Chesterfield, MO.

<sup>d</sup> Sigma Chemical Co, St Louis, MO.

<sup>e</sup> Hain Pure Food Co, Los Angeles, CA.

<sup>f</sup> Intox Products, Albuquerque, NM.

<sup>g</sup> Gelman Scientific Inc, Ann Arbor, Mich.

<sup>h</sup> Beckman L55800 scintillation counter, Beckman Instruments Inc, Irvine, CA.

Fig 1 - Adrenal gland of T-2 mycotoxin-exposed castrated female mouse receiving vehicle only (group 2). Note the zonal necrosis (between arrowheads) in the inner zona fasciculata; 24-hr postexposure; H&E stain; X 25.

Fig 2 - Adrenal gland of T-2 mycotoxin-exposed intact female mouse receiving vehicle only (group 2). Note coagulative necrosis of parenchymal cells with sparse numbers of neutrophils in the zona fasciculata; 24-hr postexposure; H&E stain; X 64.