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Toxicity of T-2 Toxin, a *Fusarium* Mycotoxin, to Alveolar Macrophages *in Vitro*

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The exposure of agricultural workers to dust particulates, which may be contaminated with common fungi and/or their toxic metabolites, is an occupational health concern. To assess the cytotoxic properties of T-2 toxin, rat alveolar macrophages (AM) were exposed to T-2 in tissue culture. Determinations of cell viability, cell number, and viability index indicate that T-2, a trichothecene mycotoxin, is highly toxic to AM. The concentrations of T-2 toxin required to decrease these parameters by 50% at 20 hr were 8.93, 0.33, and 0.89 μM, respectively. The effect of T-2 toxin on AM cell volume was dose dependent, with cultures containing 0.1 μM T-2 being significantly different than controls. The data show that T-2 toxin causes significant AM shrinkage. The amount of chromium released from preloaded AM after 18 hr of incubation was significantly different in culture containing 0.01 μM or greater T-2 toxin. The percentage of chromium released was dose dependent and parallel to the amount of cell death occurring in each culture. Scanning electron microscope examination of AM treated 20 hr with 0.1 μM T-2 toxin demonstrated detachment of pseudopodia, cellular blebing, smoothing of membrane processes, and finally cell lysis. Thus, the data clearly demonstrate that T-2 toxin is cytotoxic for rat alveolar macrophages *in vitro* and suggest the possibility of a respiratory hazard to agricultural workers.

INTRODUCTION

Previous investigations have demonstrated that respiratory disorders may result from exposure to grain dust (doPico *et al.*, 1980; Schilling, 1980). Pulmonary manifestations include chronic cough with sputum production, wheezing, and shortness of breath. However, few attempts have been made to catalog the potential etiologic agents and assess their relative health importance.

Grain dust is a heterogeneous substance that may include particles from numerous cereal grains (wheat, barley, rye, oat, and corn) and may contain a large number of contaminants including silica, fungi and their metabolites, bacterial endotoxins, insects, mites, mammalian debris, and various chemical additives such as pesticides and herbicides. Mycotoxin contamination of various grain products has been well documented (Morooka *et al.*, 1972; Lee and Chu, 1981). Recently, Sorenson *et al.* (1981) reported high levels of aflatoxin associated with the respirable fraction of a corn dust aerosol generated during dumping corn from a truck. To date, virtually no information exists on the human health significance of inhaling mycotoxin-contaminated dusts.

T-2 toxin, a mycotoxin produced by several *Fusarium* species, is widely distributed in nature. *Fusarium* contaminates a great number of field crops such as

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maize, wheat, and oat (Ueno, 1977b). The toxic metabolites are responsible for a human disease outbreak (alimentary toxic aleukia) and have also been implicated as the causative agent in many animal intoxications (Ueno, 1977a). Trichothecenes are cytostatic for unstimulated and mitogen-stimulated lymphocytes (Lafarge-Frayssinet *et al.*, 1979), fibroblasts (Oldham *et al.*, 1980), neoplastic cells such as HeLa cells (Sato *et al.*, 1975), and transformed intestinal cells (Morel-Chany *et al.*, 1980). In addition Lafarge-Frayssinet *et al.*, (1981) have shown that T-2 toxin induces severe damage to rat splenic cells *in vitro*. T-2 toxin induces the depletion of murine thymus (Lafont *et al.*, 1977), inhibits the synthesis of anti-sheep red blood cell antibodies, and prolongs the period required for skin graft rejection (Rosenstein *et al.*, 1979). They strongly inhibit protein and DNA synthesis (Ueno, 1977b). The initiation step of translation is impaired (Cundliffe *et al.*, 1974) as well as the termination reaction (McLaughlin *et al.*, 1977). Cannon *et al.* (1976) have demonstrated that T-2 toxin inhibits peptide bond formation on ribosomes at the level of the peptidyl transferase catalytic center. Recently it has been claimed that the USSR has been engaging in chemical warfare involving mycotoxins of the trichothecene family (Marshall, 1982).

The purpose of this investigation is to examine the toxicity of T-2 toxin on rat alveolar macrophages *in vitro*. Pulmonary macrophages maintain the sterility of the lower respiratory tract by ingesting and inactivating living and nonliving particulate material to which the lung is exposed. Additional functions of these cells include regulation of T-lymphocyte proliferation, provision of T-helper activity for antibody production, and production of mediators of cellular immunity. Thus, damage to AM[1] by T-2 toxin could lead to serious pulmonary and/or systemic damage.

MATERIALS AND METHODS

T-2 Toxin Preparation

The mycotoxin T-2 (3-hydroxy-4,15-diacetoxy-8-[3-methylbutyryloxy]-12,13-epoxy-Δ^9-trichothecene) was dissolved in 100% DMSO (Pierce Chemical Co., Rockford, Ill.) at a concentration of 10 mM and stored at −20°C. Substocks were prepared by performing 10-fold dilutions in 10% DMSO. The final concentration of DMSO in all experimental cultures, including controls, was 0.1%. Diacetoxyscirpenol (DAS) was prepared and handled as described above. T-2 toxin and DAS were purchased from Calbiochem, La Jolla, California. The purity of these preparations was tested by thin-layer chromatography (TLC). No contaminant spots were observed after charring with sulfuric acid.

Alveolar Macrophage Isolation and Culture

Alveolar macrophages were harvested from male Long–Evans hooded rats by tracheal lavage according to the method of Myrvik (1961). Rats were anesthetized

[1] Abbreviations used: AM, alveolar macrophages; DMSO, dimethyl sulfoxide; DAS, diacetoxyscirpenol; HBSS, Hanks' balanced salt solution; HI-FBS, heat-inactivated fetal bovine serum; P, penicillin; S, streptomycin; H, heparin; NVD, normal volume distribution; MCV, mean cell volume; SEM, scanning electron microscopy.

by intraperitoneal injection with sodium pentobarbital, 0.2 mg/g body wt (Butler Co., Columbus, Ohio), and exsanguinated by cutting the abdominal aorta. The lungs from each rat were lavaged approximately six times with a total of 60 ml of prewarmed lavage fluid, Hanks' calcium–magnesium-free balanced salt solution (HBSS). The cells were separated from the lavage fluid by centrifugation at 500*g* for 10 min. Cells from several animals were pooled and washed with phosphate-buffered saline (PBS). Supplements added to the medium included: 2% heat-inactivated fetal bovine serum (HI-FBS); penicillin (P), 100 units/ml; streptomycin (S), 100 μg/ml; and heparin (H), 20 units/ml. Medium and supplements were obtained from Grand Island Biological Company, Grand Island, New York. A hemocytometer or a Coulter counter (Coulter Instrument Co., Hialeah, Fla.) was employed to determine the cell concentration.

Aliquots of the cell suspension were added to the various tissue culture vessels so that the cell monolayer concentration would equal 2.5×10^5 cells/cm^2. Tissue culture vessels employed for the various experiments included 35-mm tissue culture plates (Falcon, Oxnard, Calif.) and Linbro tissue culture plates (Linbro, Hamden, Colo.). AM monolayers in medium 199 (2% HI-FBS, H, P, S) were incubated at 37°C in 5% CO_2 to allow adherence of AM. After 2 hr of incubation the monolayers were rinsed two times with PBS to remove nonadherent cells. The monolayers were then incubated with medium 199 (10% HI-FBS, P, S) until needed for experiments.

Determination of Alveolar Macrophage Characteristics

The viability of both preadhered and cultured macrophages was determined by assessing the ability of macrophages to exclude trypan blue (Phillips, 1973).

The cell volumes of preadherent and cultured AM were routinely determined using a Coulter Model Z_B electronic cell counter interfaced with a Channelyzer II cell-sizing attachment. Aliquots of the cell suspension were suspended in Isoton (Curtin Matheson, Cleveland, Ohio). Preadherent cells were suspended directly and cultured cells were trypsinized and then added to the Isoton. Mean cell volumes (MCV) were expressed in cubic micrometers.

The proportion of esterase-positive cells present in both preadherent and cultured AM was determined employing the method described by Wier (1978). Aliquots of both were centrifuged onto glass slides with a cytocentrifuge at 200*g* for 15 min. The cells were fixed with citrate–acetone–methanol fixative for 30 sec at room temperature and then washed extensively. The samples were stained for 15 min at 37°C with α-naphthyl acetate substrate (Sigma Chemical Co., St. Louis, Mo.) to determine the number of AM positive for nonspecific esterase activity. The cells were counterstained with Myers hematoxylin solution (Sigma) for 5 min and then examined microscopically.

Viability, Cell Number, and Viability Index Assay

To determine the toxic effects of T-2 toxin and DAS on rat alveolar macrophages, an in-depth viability study was performed using the methods described by Waters *et al.* (1975a). AM monolayers were treated with DAS and T-2 toxin concentrations of 10^{-3} to 10 μM for 20 hr. After treatment, the medium containing

unattached cells was collected and retained. The attached cells were removed by incubating the AM monolayers in 2 ml of 1% trypsin (Gibco) for 3 min. The original culture medium and the trypsinized suspension were combined and counted with a hemocytometer. For counting, 0.4 ml of each cell suspension was added to 0.1 ml of a 0.4% trypan blue solution.

The cell viability was estimated by light microscopy on the basis of trypan blue exclusion (Phillips, 1973). For both treated and untreated cultures the number of viable and nonviable cells was tabulated. Cell viability was obtained by dividing the number of viable cells by the total number of intact cells. The cell number of each culture was determined by counting the total number of intact cells per treated culture and dividing by the number of intact cells per control culture. Thus, the cell number of control cultures would equal one. Viability index value was obtained by multiplying the viability ratio by the cell number ratio (Waters, 1975b). The viability index value was the net number of viable cells (or conversely, net cell death) as a percentage of control. Arc-sine transformation and linear-regression calculations were done with the Parklawn computer statistical analysis system.

Determination of Cell Volume Distribution

AM monolayers were incubated with various concentrations of T-2 toxin for 18 hr. After incubation the culture medium was aspirated and discarded. To harvest the macrophages 250 μl of 0.25% trypsin–EDTA (Gibco) containing 0.06 mg/ml DNase (Sigma) was added to each culture for 5 min at 37°C. Each well was carefully scraped with a plastic policeman. The cell suspension was then added to 10 ml of Isoton in Coulter counter vials. Cell volume determinations for both control and T-2-toxin-treated cultures are the result of three experiments done in triplicate. To determine the mean cell volume (MCV) values the equation MCV = (channel No. + base channel threshold)/(threshold factor) was employed (Castranova *et al.*, 1979). By assigning an arbitrary distribution for normal macrophage volume of 600–2500 μm^3 the percentage of both control and T-2-toxin-treated AM which fell into this assigned normal volume distribution (NVD) was determined.

Chromium Release Assay

Isolated AM were washed two times with PBS and resuspended in medium 199 (10% HI-FBS,P, S) at a cell concentration of 1.0×10^7 cells/ml. The AM were labeled with radioactive sodium chromate (New England Nuclear, sp act 296.3 Ci/g) at a 100 $\mu Ci/10^7$ cell ratio (Sanderson, 1976). The AM were incubated with the labeled chromium at 37°C in 5% CO_2 for 45 min. Following incubation the AM were washed three times with HBSS. The supernatant of the final wash was assayed to assure that nonincorporated radioactivity was removed. The AM were resuspended in a small volume of medium 199 and counted, the cell concentration was adjusted to 2.5×10^6 cells/ml, and 0.1 ml of the labeled cell suspension was added to a series of 10 × 75-mm test tubes. Each tube then received 0.1 ml of medium 199 containing the appropriate concentration of T-2 toxin. Control and treated AM were incubated for 18 hr at 37°C in 5% CO_2 on a rotary shaker.

Spontaneous release of labeled chromium from untreated cultures was monitored after 18 hr. Total release of chromium from AM was obtained with 0.1% Triton X-100. To assay chromium release the culture tubes were centrifuged at 200*g* for 10 min. Culture supernatant (100 μl) was removed and counted in a Packard Tri-Carb gamma counter. The percentage chromium release equals the chromium release of experimental cultures divided by the total release value. Both values were corrected for spontaneous release.

Morphological Study of Treated and Untreated Alveolar Macrophages

AM monolayers were prepared on round 15-mm plastic tissue culture coverslips (Lux Scientific Co., Thousand Oaks, Calif.) and exposed to the appropriate T-2-toxin concentration for 18 hr. For scanning electron microscopy (SEM) the AM were fixed for 48 hr in a 2% buffered glutaraldehyde solution at room temperature with or without postosmication. A 1% osmium tetroxide solution in 0.2 M *syn*-collidine buffer was employed. The fixative and buffer were pH 7.2 ± 0.1. The cells were dehydrated through a graded series of alcohols (10%–100% ethanol with 10% gradation). Specimens were subsequently taken through graded solutions of alcohol–amyl acetate in preparation for critical point drying. Critical point drying was performed using a Denton DCO-1 critical point drying apparatus and liquid CO_2 followed by coating the cells with a thin coating (≈200 Å) of gold/palladium in a Polaron E5100 series II cool sputter (Leake and Wright, 1979; Miller, 1979).

RESULTS

Characteristics of Alveolar Macrophages

The cellular characteristics of isolated and cultured rat alveolar macrophages are presented in Table 1. The percentage viability of both preadherent and cultured AM was consistently greater than 95%. The cell volume values of cultured AM were greater than preadherent macrophages presumably due to their ability to adhere and spread on the tissue culture apparatus. If one assumes that macrophages are spherical, the mean diameter can be calculated to be approximately

TABLE 1
CHARACTERISTICS OF PURIFIED ALVEOLAR MACROPHAGES FROM LEH RATS

Cell	Percentage of purified AM positive by			
	Viability[a]	Cell volume[b]	Esterase[c]	Phagocytosis[d]
Preadhered	96.4(±0.67)[e]	1119.3(±46.3)	93.6(±1.7)	—
Cultured macrophages (20 hr)	95.9(±0.15)	1279.5(±102.9)	98.7(±2.4)	92.3(±2.09)

[a] Percentage viability as determined by trypan blue exclusion.
[b] Cell volume values as determined by a Coulter Model Z_B electronic cell counter interfaced with a Channelyzer II cell-sizing attachment. Cell volume units are in cubic micrometers.
[c] Cells staining positive for nonspecific esterase.
[d] Cells actively phagocytizing zymosan particles.
[e] Values represent the mean ± standard deviation.

12.8 μm. This value for the AM diameter is similar to that reported by Castranova *et al.* (1979). Of the preadherent macrophages, 93.6% were positive for esterase, whereas approximately 98.7% of the cultured macrophages were positive. The percentage of AM capable of phagocytizing zymosan particles was 92.3%. The data indicate that the cultured cells are a viable and nearly homogeneous population of AM.

Viability Determinations of T-2-Toxin-Treated Alveolar Macrophages

The effect of T-2 toxin on viability, cell number, and viability index is presented in Fig. 1 (A–C, respectively). The viability of control cultures was 96.0%, whereas cultures containing T-2 toxin at concentrations of 0.001 and 0.01 μM showed a viability of 93.8 and 89.8%, respectively. The viability was reduced to 51.9 and 47.7% in cultures incubated with 1 and 10 μM T-2 toxin. At the highest concentrations of T-2 toxin tested, the viability of the cells remaining was approximately 50%.

Cell number values (Fig. 1) depict the ratio of intact cells in experimental cultures to the number of intact cells in control cultures. Cultures containing T-2-toxin concentrations of 0.001 and 0.01 μM, which had slightly reduced viability values, had cell number values of 79.8 and 69.0%. With 1 and 10 μM cultures (approximately 50% viability) the cell number values were 30.3 and 29.4%, respectively.

A better indication of the cytotoxic effects of T-2 toxin on AM is provided by the viability index value (Waters *et al.*, 1975b). Viability index values (Fig. 1) are the product of the cell viability ratio and cell number ratio. The viability index parameter represents the net number of viable AM. A dose-dependent response was obtained.

Since the observed response varied between 5 and 100% of the control response, and since the sample data could be considered as observations from a binomial population, an arc–sine transformation was used for regression analysis (Waters *et al.*, 1975b).

DAS was employed for comparison of T-2 toxin's cytotoxicity. Viability, cell number, and viability index values were calculated following 20 hr exposure. With the use of the predicted lines obtained from the viability, cell number, and viability index transformation data, it was possible to predict the concentrations of T-2 toxin and DAS causing reduction in viability index to 50% at 20 hr (Table 2). The T-2-toxin and DAS concentrations needed to reduce viability index to 50% after 20 hr were 0.089 and 0.239 μM, respectively, indicating that T-2 toxin is more cytotoxic than DAS.

Cell Volume Determinations of T-2-Toxin-Treated Alveolar Macrophages

Cell volume determinations of AM treated with T-2 toxin for 18 hr are presented in Table 3. The MCV values decreased in cultures with increasing concentrations of T-2 toxin. The MCV values of AM cultures with 0.1 μM T-2 toxin are significantly different than controls. Cultures containing 1 and 10 μM T-2 toxin were not assayed due to excessive cell death occurring in these cultures.

Normal volume distribution values of control and treated AM are also pre-

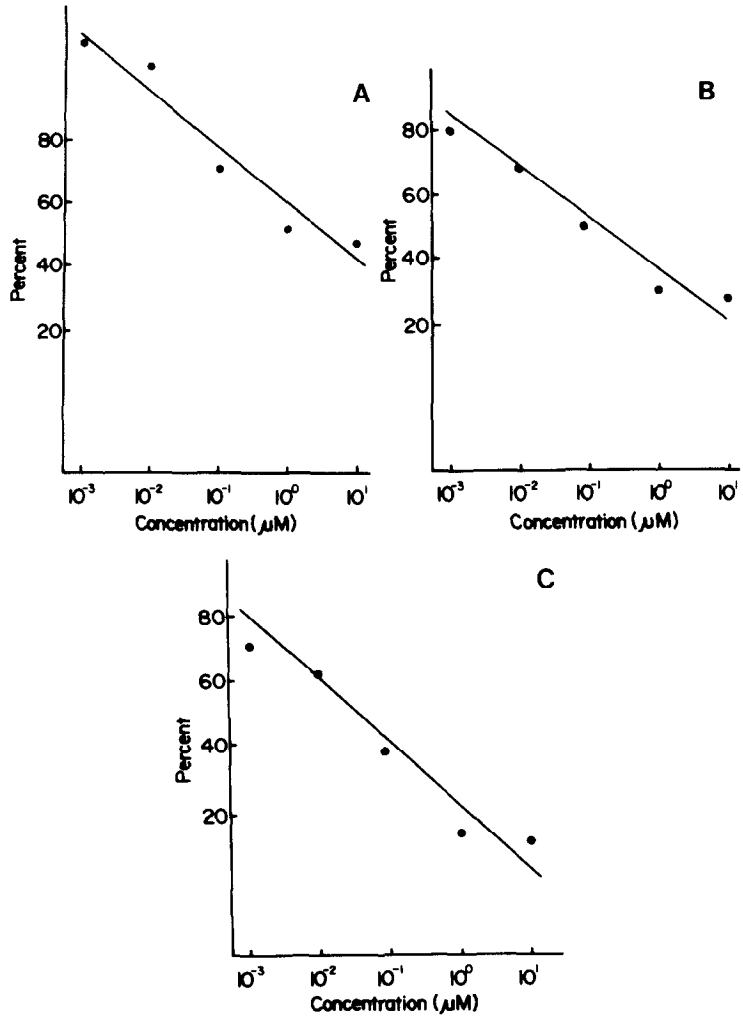

FIG. 1. Effect of T-2 toxin on viability (A), cell number (B), and viability index (C) at 20 hr. Note arc-sine scale on the ordinate.

sented in Table 3. The assigned NVD is 600–2500 μm^3. A decreasing trend in NVD values is evident with increasing concentrations of T-2 toxin. NVD values of cultures containing 0.01 and 0.1 μM T-2 toxin were significantly different than controls. The normal volume distribution graphs of cultures representing control and 0.01 and 0.1 μM T-2 toxin are presented in Fig. 2. The graph demonstrates a shift in AM volume distribution after incubation of AM with increasing concentrations of T-2 toxin.

Chromium Release Determinations of T-2-Toxin-Treated Alveolar Macrophages

Chromium release determinations of AM treated with T-2 toxin for 18 hr are presented in Table 3. The amount of ^{51}Cr released from AM cultures containing

TABLE 2
CONCENTRATIONS OF T-2 AND DAS CAUSING REDUCTION IN VIABILITY, CELL NUMBER, AND VIABILITY INDEX TO 50% AT 20 hr

Mycotoxin	Concentration of mycotoxin (μM)[a]		
	Viability	Cell number	Viability index
T-2	8.93 (1.64–48.7)	0.330 (0.017–0.806)	0.089 (0.009–0.30)
DAS	3.37 (0.568–20.0)	0.686 (0.024–0.763)	0.239 (0.016–0.472)

[a] Values shown represent the mean, $n = 9$. Numbers in parentheses represent 95% confidence intervals.

T-2 toxin concentrations of 0.01–10 μM were significantly different than controls and showed a dose-dependent response. However, cultures containing 0.001 μM T-2 toxin were not significantly different from controls. These data correlate well with the lethality observed with T-2 toxin in the initial viability–cell number experiments.

Morphological Study of Treated and Untreated Alveolar Macrophages

The intricate surface structure of cultured control macrophages viewed by scanning electron microscopy is shown in Figs. 3A and 4A. Numerous membrane processes can be seen projecting upward as well as attaching the cell to the glass coverslips. The normal rat AM exhibited a characteristic ruffled membrane surface with fingerlike pseudopodia. Generally the cells remained rounded, although moderate cell spreading was sometimes seen.

The membrane changes of AM exposed to T-2 toxin contrasted strikingly when compared to control AM. The severe damage to macrophages caused by a 20-hr exposure to 0.1 μM T-2 toxin is presented in Figs. 3B–D. Most of the cells have

TABLE 3
CELL VOLUME AND ^{51}Cr-RELEASE CHANGES FOLLOWING 18 hr OF INCUBATION OF ALVEOLAR MACROPHAGES WITH T-2 TOXIN

T-2 toxin (μM)	Cell volume		^{51}Cr release	
	MCV (μm^3)[a]	NVD (%)[b]	cpm	%
Control	1236.2(±101.5)[c]	96.8(±1.00)	6473.1(±949.1)	—
0.001	1182.9(±138.5)	96.6(±1.03)	6575.0(±529.0)	0.38
0.01	1107.5(±98.0)	94.5(±1.76)[d]	10202.8(±350.1)	14.1[d]
0.1	1002.3(±26.5)[d]	71.6(±3.34)[d]	14027.8(±259.4)	28.6[d]
1.0	ND[e]	ND	17394.5(±918.3)	41.3[d]
10.0	ND	ND	18758.7(±483.3)	46.4[d]

[a] MCV, mean cell volume.

[b] NVD, normal volume distribution, percentage of AM falling in a normal volume distribution of 600–2500 μm^3.

[c] Values shown represent the mean ± standard deviation.

[d] Values are significantly different than controls, $p < 0.005$.

[e] Not determined.

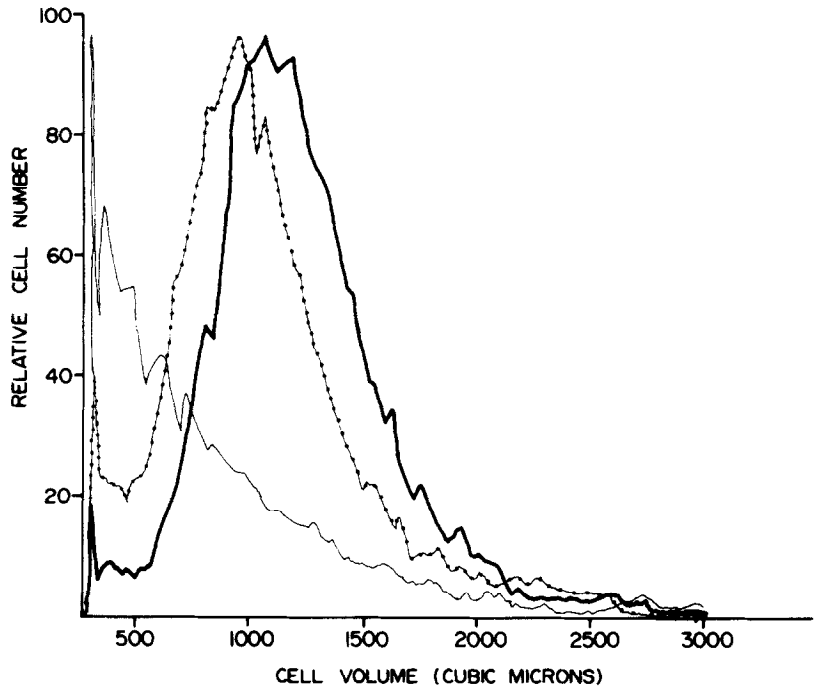

FIG. 2. Alveolar macrophage cell volume after 18 hr of treatment at 0.01 μM (-●-●-●-) and 0.1 μM T-2 toxin (—). Control NVD (—) is included for comparison.

completely lost their surface processes and many have developed bleblike structures or appeared to have been broken open. Also, some treated AM exhibited an unusually smooth membrane surface. Figure 4 depicts the various types of cellular damage observed at higher magnification. Figure 4B shows the retraction of the normally extended pseudopodia, whereas Fig. 4C shows the appearance of numerous bleblike structures following treatment with T-2 toxin. Smoothing of the plasma membrane is evident in Fig. 4D.

DISCUSSION

Viability determinations after exposure of AM to submicromolar concentrations of T-2 toxin for 20 hr demonstrated that the toxin was cytotoxic at determinable concentrations and that the effect of T-2 toxin on AM viability, cell number, and viability index was dose dependent. The viability index parameter developed by Waters *et al.* (1975a,b) at the Environmental Protection Agency (EPA) to assess cytotoxicity of environmental compounds is useful when cell death proceeds via mechanisms which result in differing degrees of cell lysis. For example, the viability of cultures containing 0.1 μM T-2 toxin was 73.9% after 20 hr of incubation; however, the cell number in those cultures was only 50.8% of controls. At 0.001 μM T-2 toxin the viability was 93.8%, yet the cultures contained 21% fewer cells than the control cultures which had a viability of 96%. One explanation for this is that T-2 toxin causes rapid cell lysis.

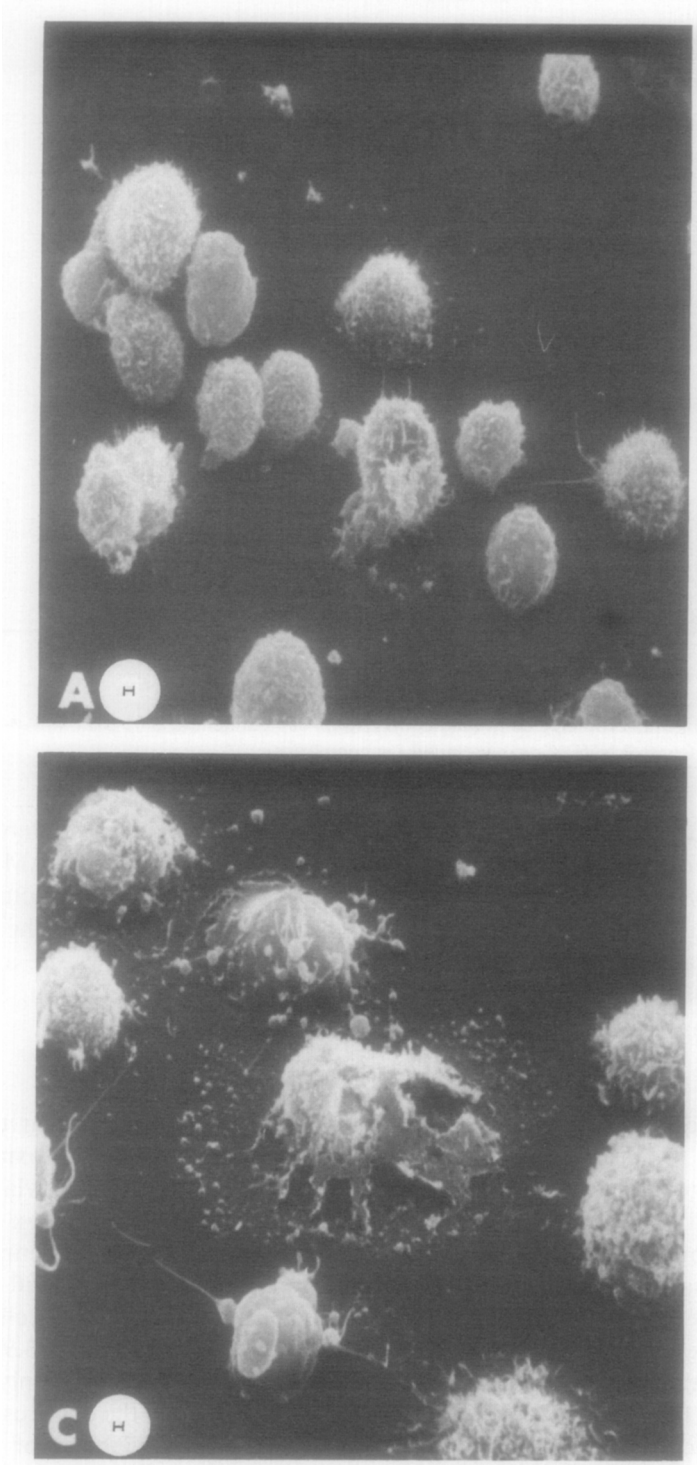

FIG. 3. Scanning electron micrographs of alveolar macrophages at 20 hr. Control (A) and 0.1 μM T 2 toxin (B–D). Bar represents 1.0 μM.

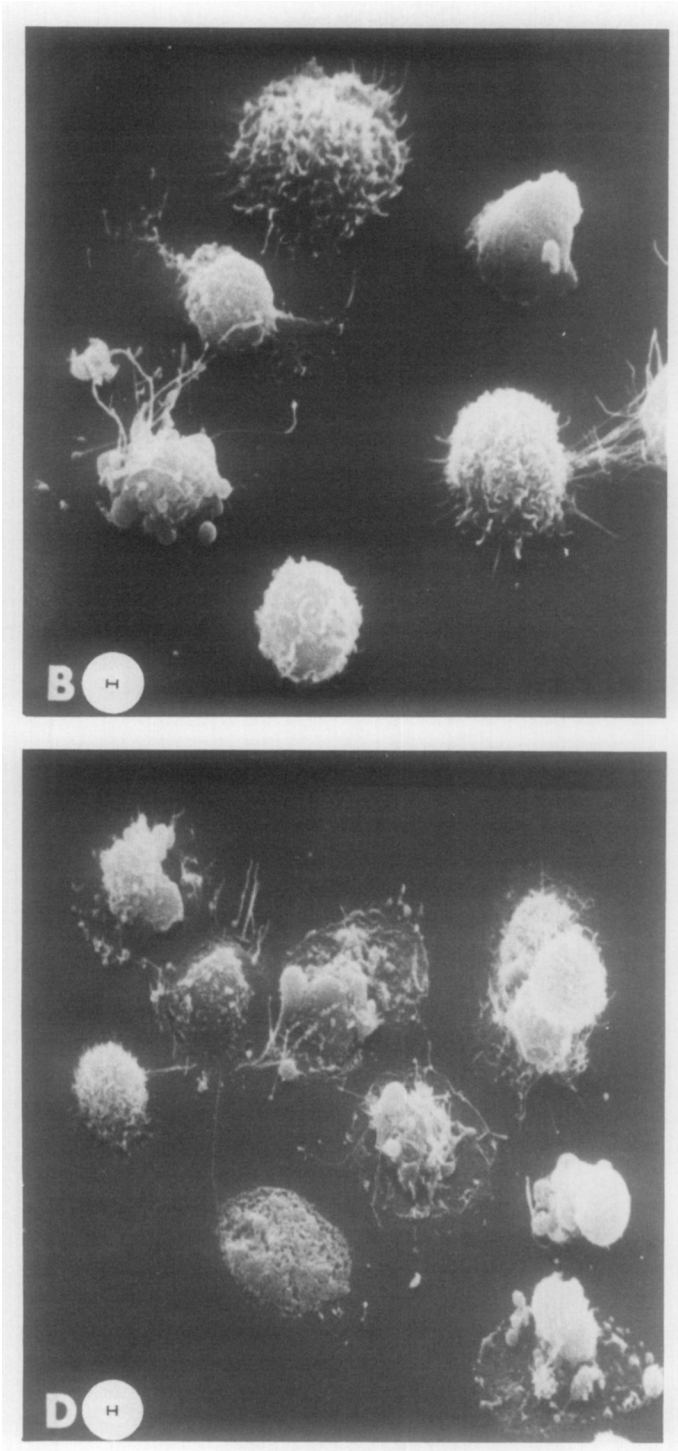

FIG. 3—*Continued*

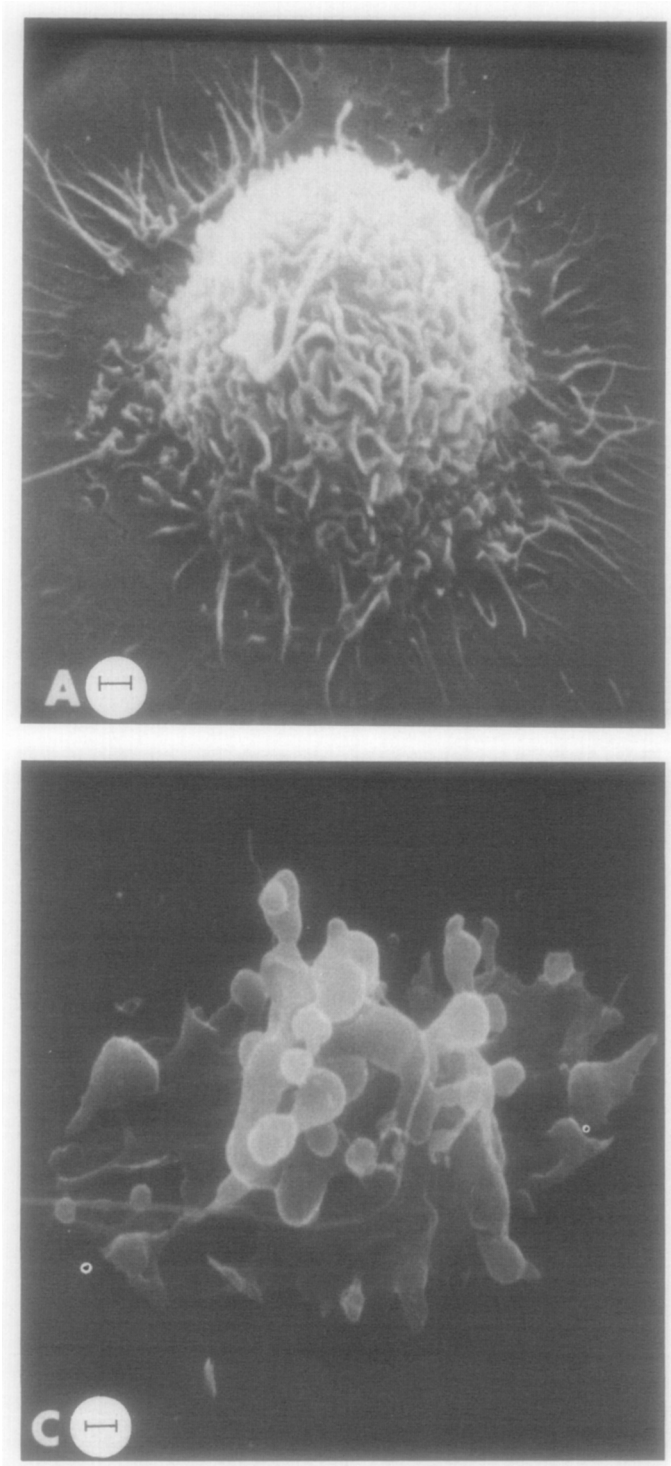

FIG. 4. Scanning electron micrographs of alveolar macrophages at 20 hours. Control (A) and 0.1 μM T-2 toxin (B–D). Bar represents 1.0 μM.

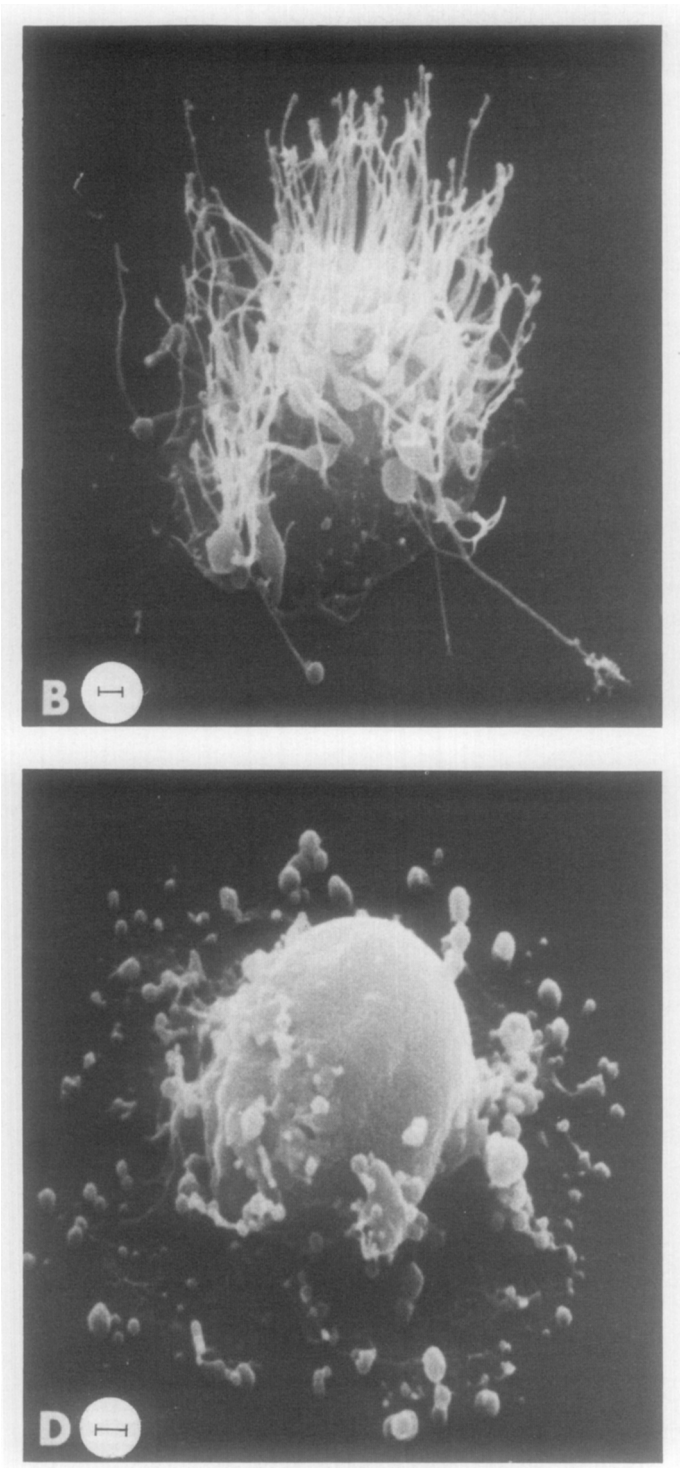

FIG. 4—*Continued*

Arc-sine transformations of the viability, cell number, and viability index data allow comparison of respective slopes and allow prediction of a ED_{50} at 20 hr. DAS was chosen for comparison because it is identical to T-2 toxin in structure except it lacks the isovaleryl group at C8 and has been shown to be less toxic than T-2 toxin in various cytotoxicity assays (Ueno, 1977b; Ueno *et al.*, 1969). T-2 toxin is more cytotoxic for rat AM than DAS (viability index). If one allows a comparison to some EPA studies done with other environmental agents, it has been reported that the amount of Cd^{2+} needed to reduce the viability index of rabbit AM to 50% in 20 hr was 0.082 mM as compared to 0.089 μM T-2 toxin (Waters *et al.*, 1975a). Thus, T-2 toxin is approximately 1000-fold more inhibitory than Cd^{2+} in this system.

AM incubated 18 hr with T-2 toxin displayed a dose-dependent decrease in their cell volume values. Cultures containing 0.1 μM T-2 toxin were significantly different than control cultures in their cell volume and NVD values. Figure 2 depicts a shift in AM cell volume distribution following 18 hr of incubation with 0.01 and 0.1 μM T-2 toxin.

Treatment with T-2 toxin also resulted in leakage of chromium from the cells. When AM were treated for 18 hr the amount of chromium released from cultures containing as little as 0.01 μM T-2 toxin were significantly different than control cultures. An increase in chromium leakage was observed with increasing concentrations of T-2 toxin. The amount of chromium released from the treated AM parallels the amount of cell death which occurred in these cultures.

The scanning electron micrographs demonstrate surface morphologic alterations in the alveolar macrophage *in vitro* that reflect varying degrees of cell damage. Most of the cells have completely lost their surface membrane features and have developed bleblike structures and many lysed cells are also evident. Waters *et al.* (1975a) have suggested the following sequence of events in AM damage *in vitro:* (a) retraction of normally extended pseudopodia; (b) appearance of bleblike surface structures; (c) smoothing of the plasma membrane; and (d) final destruction of the cellular architecture. T-2 toxin produced a similar sequence of events (Figs. 4B–D). Miller (1979) has reported that macrophages isolated from the lungs of animals exposed to quartz particles by inhalation also exhibited a loss of characteristic filopodia and the appearance of bleblike structures on the surface of the cell. T-2 toxin also had deleterious effects on AM nucleus (pycnosis, karyorrhexis, and karyolysis) which were observed by light microscopy examination (data not shown).

The results of this investigation clearly demonstrate that submicromolar concentrations of T-2 toxin are cytotoxic for rat AM. The major health question is: Does T-2 toxin pose an environmental hazard for agricultural workers exposed to mycotoxin-contaminated grain dust? Grain dust samples of various kinds have been shown to be contaminated with various mycotoxins at greater than parts per million concentrations (Eppley, 1975; Sorenson *et al.*, 1981). It has also been shown that mycotoxins can be associated with the respirable fractions of a grain-generated aerosol (Sorenson *et al.*, 1981). The question whether agricultural workers are exposed to significant amounts of these mycotoxins *in vivo* is unknown. Based on known levels of trichothecene contamination in grain (Hsu *et*

al., 1972; Mirocha *et al.*, 1979; Morooka *et al.*, 1972), the concentration of dust in various parts of grain elevators (Farant and Moore, 1980; Yoshida and Maybank, 1980), and the minute volume of an average adult male at rest (Comroe, 1974), one can estimate that a grain elevator worker could inhale > 1 μg of T-2 toxin in an 8-hr day.

Further research is needed to clarify the significance of mycotoxin contamination of grain and determine its relation to pulmonary disease seen in workers exposed to grain aerosols. In addition other mycotoxins known to contaminate grain need to be investigated.

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