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Cellular effects of T-2 mycotoxin on two different cell lines

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The effects of T-2 toxin on protein synthesis, respiratory chain activity of the mitochondria, cell lysis and toxin-cell binding were compared in the toxin-sensitive bovine kidney cell line (MDBK) and in the toxin-resistant Chinese hamster ovary cell line (CHO). Protein synthesis and mitochondrial activity were 10-fold less sensitive in CHO cells as compared to MDBK (50% inhibition = 10–15 ng/ml vs. 1–1.5 ng/ml, respectively). Lytic activity, as determined by release of ^{51}Cr from cells incubated at 4°C, was not detected at 5 h or 24 h in either of the cell lines. However, at 24 h, CHO cells released less ^{51}Cr than non-toxin-exposed controls, indicating that some membrane interaction does occur. Both cell lines equally bound [^{3}H]T-2 toxin at 4°C. At 37°C, the MDBK cells take up twice as much [^{3}H]T-2 toxin at 2 h and 6 h. These results indicate that T-2 toxin mediates a number of effects on the cell at the level of the membrane, protein synthesis and probably mitochondrial activity toward 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction.

Introduction

A number of mycotoxins have been isolated from animal feeds and implicated in food-borne intoxications of humans and farm animals [1–3]. T-2 toxin, the most potent of the trichothecene mycotoxins [3], has been extensively studied and has been shown to exert multiple effects on mammalian cells. The primary mode of action of T-2 toxin is the inhibition of protein synthesis, but exposure of the cell to the mycotoxin also results in decreased DNA and RNA synthesis [4,5], inhibition of mitochondrial function [6–10], breaks in the DNA chain [11] and changes in membrane morphology and osmotic fragility [12–16]. T-2 toxin exerts its lethal effects on a wide range of eukaryotic cells, including mammalian [9,10,17], insect [10,18] and yeast [7,8]. Cell lines from most animal species exhibit similar dose responses to T-2 toxin [17], but several cell lines differ in their susceptibility to the toxin's lethal effects [10]. CHO cells require a higher dose of toxin to be killed than most other cell lines and conversely the bovine cell line MDBK is affected by much lower toxin concentrations [10]. We conducted experiments to examine the effects of T-2 toxin on these two cell lines. Protein synthesis, mitochondrial activity, cell lysis and toxin binding were examined and significant differences were noted.

Abbreviations: CHO, chinese hamster ovary cell line; DME, Dulbecco's Modified Eagle's medium; MDBK, Madin Darby bovine kidney cell line; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate-buffered saline; $TCID_{50}$, tissue culture 50% inhibitory dose. Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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Materials and Methods

T-2 Mycotoxin. T-2 mycotoxin (more than 99% pure) was purchased from Mycolabs, Chesterfield, MO, and was maintained as a 2 mg/ml stock in dimethylsulfoxide at 4° C. Serial 2-fold dilutions of the stock were made in Dulbecco's Modified Eagle's (DME), high glucose (Hazleton Dutchland, Denver, PA.) base media containing 0.1% dimethylsulfoxide.

Cell lines. The bovine kidney cell line MDBK (CCL 22), was obtained from American Type Culture Collection, Beltsville, MD and the chinese hamster ovary cell line CHO, was provided by Dr. Michael Misfeldt, Department of Microbiology, University of Missouri, Columbia, MO. The tissue culture cells were grown in DME, high glucose, supplemented with 5% fetal bovine serum, L-glutamine (4 mM), nonessential amino acids (1 × concentration), and sodium pyruvate (1 mM). Gentamicin (100 μg/ml), penicillin (50 U/ml) and streptomycin (50 μg/ml) were added as the antibiotics. The cells were maintained as stock cultures in 80 cm^2, 260 ml flasks (Nunc 153732, Hazleton Dutchland) at 37° C in a humidified 7% CO_2 atmosphere. Cells were detached from the flask by 2–5 min incubation with trypsin (0.25% w/v in phosphate-buffered saline (PBS), 0.01 M (pH 7.2)), centrifuged and resuspended in culture medium to appropriate density for assay. Cells were enumerated with a hemocytometer.

T-2 Toxin dose effects. Stock cells were resuspended to $2 \cdot 10^5$ cells/ml in culture medium and 100 μl aliquots were dispensed into 96-well tissue culture trays (Nunc 167008, Hazleton Dutchland, Denver, PA). 50 μl aliquots of serial 2-fold T-2 toxin dilutions were transferred to quadruplicate cell cultures. A toxin-control group received 50 μl of diluent, DME/0.1% dimethylsulfoxide. All cultures were incubated for 48 h and the effects of the toxin on either CHO or MDBK MTT reduction by mitochondrial enzymes or protein synthesis were determined as described below. Results are representative of multiple experiments.

Cell mitochondrial activity assay. $2 \cdot 10^5$ CHO or MDBK cells/ml in 100 μl were seeded into individual wells of a 96-well tissue culture tray. The cells were incubated at 37° C in 7% CO_2 and quadruplicate cultures were assayed for MTT reduction at 4 h, 8 h, 24 h and 48 h following the start of the experiment. The relationship of incubation time to MTT reduction was analyzed by linear regression and plotted using the Sigma Plot statistical plotting software (Jandel Scientific, Sausalito, CA).

MTT reduction assay. Following the specified incubation time, the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma catalog No. M2128), dissolved in PBS at 5 mg/ml, filter sterilized, and maintained at 4° C in the dark was added to each well of the tissue culture tray at the rate of 10 μl per 100 μl medium. Fresh MTT solution was prepared daily. After 4 h incubation, the media was removed and 100 μl acid-isopropanol (0.04 M HCl in isopropanol) was added to all wells. The samples were thoroughly mixed by repeated pipetting to dissolve the blue formazan product formed by mitochondrial enzyme activity. The absorbance was determined with a Dynatech MR580 Microelisa reader (Dynatech Laboratories, Inc., Chantilly, VA) using a test wavelength of 570 nm and a reference wavelength of 630 nm and blanked on a well containing alcohol solvent. Means ± standard errors were calculated on the absorbance readings of each dilution group. When applicable, the means were converted to a percentage of the control response by the formula: % control response = (response of toxin group/response of control group) · 100. These results were computer plotted using the Sigma Plot software mentioned above and 50% inhibition values ($TCID_{50}$) were derived from the curves.

Protein synthesis inhibition. Inhibitory effects of T-2 toxin on protein synthesis were analyzed using a modification of a protein synthesis inhibition method previously described [17]. 48-h cultures were pulsed for 4 h with 1 μCi tritiated L-leucine ([^{3}H]Leu, 146.7 Ci/mM, New England Nuclear, Boston, MA). The medium was removed by inverting the 96-well tissue culture trays, and 100 μl of 0.1 M NaOH was added to each well for 5 min to detach and solubilize the cells. The cultures were harvested and adsorbed onto filter strips (Skatron, Inc., Sterling, VA) with cold 10% trichloroacetic acid (Fisher Scientific, Fair Lawn, NJ) using a Skatron 7019 cell harvester. The filters were washed twice with cold 5% trichloroacetic

acid, once with cold ethanol/acetone (1:1, v/v) and once with cold acetone and then allowed to dry. All filter washes were conducted in glass dishes for 5 min using 250 ml per wash. Individual discs containing the adsorbed cells were detached from the filter strip with forceps and placed in 3 ml of Ready-Solv HP/B (Beckmann Instruments, Inc., Fullerton, CA) scintillation cocktail. The amount of radiolabel incorporated into the protein was determined on an LKB RACKBETA liquid scintillation counter (LKB Wallac, Turku, Finland). Means ± standard error were calculated for the [^{3}H]Leu incorporation of each dilution group. The means were converted to a percentage of the control response by the formula: % control response = (response of toxin group/response of untreated control group) · 100. These results were computer plotted using the Sigma Plot software mentioned above and $TCID_{50}$ values were derived from the curves.

Chromium release assay. CHO or MDBK cells were harvested from the stock cultures and resuspended in culture media containing 25 mM Hepes buffer (Gibco, Grand Island, NY) to a density of $3 \cdot 10^6$ cells/ml. 1 ml of cells was mixed with 90 μCi of ^{51}Cr (200–900 Ci/g, New England Nuclear, Boston, MA) and the suspension was incubated at 37° C for 1 h with occasional swirling. Following the incubation, 25 ml of DME was added to the tube and the suspension was centrifuged at 1000 rpm for 10 min. The supernatant was removed and the cells were incubated in 25 ml DME for an additional 30 min at room temperature. The mixture was centrifuged and washed in PBS until the radioactive counts in the wash approached background levels. The cells were then resuspended to a density of $2 \cdot 10^5$ cells/ml in culture medium containing 25 mM Hepes and 100 μl aliquots were dispensed to individual wells of a 96-well tissue culture tray. 50 μl of DME, 2 μg/ml T-2 toxin (100 ng final concentration), or 0.1% Triton X-100 (Sigma, St. Louis, MO) were added to the respective group and the trays were incubated at 4° C or 37° C for 4 h or 24 h. Following the specified incubation time, the trays were centrifuged at 1500 rpm for 10 min and 50 μl of supernatant was removed from each well and counted in a 1282 Compugama gamma counter (LKB Wallac, Turku, Finland). Total chromium release was obtained with the Triton X-100 and results were expressed as a percentage of this total release value (% = (experimental release/total release) · 100). Results are representative of multiple experiments.

Toxin binding assay. Tritiated T-2 toxin ([^{3}H]T-2) was synthesized by Amersham International (Arlington Heights, IL) and provided to us by the U.S. Department of Defense (Fort Detrick, MD). The label was incorporated at C-3, and the specific activity of the preparation was 14.0 Ci/mM. Stock CHO and MDBK cells were harvested from flasks using an ethylenediaminetetraacetic acid (EDTA) buffer (Na-EDTA 0.2 g/l, NaCl 8 g/l, KCl 0.2 g/l, Na_2HPO_4 1.15 g/l, KH_2PO_4 0.2 g/l and glucose 0.2 g/l). Following centrifugation, the cells were resuspended to $2 \cdot 10^5$ cells/ml in cold tissue culture media containing 25 mM Hepes and the cells were incubated at 4° C a further 2 h. 100 μl of the suspension were added to individual wells of a 96-well tissue culture tray along with 50 μl of serial 2-fold dilutions of [^{3}H]T-2 toxin. Two sets of trays were prepared and unlabeled T-2 toxin (625 ng/25 μl) was added to one set and the other set received 25 μl of the solvent control (DME/1% dimethylsulfoxide). The trays were placed on a rotator at 4° C and rotated for 5 h. The cells were then harvested using the Skatron cell harvester and assayed for bound counts via liquid scintillation spectrometry as specified above. In a second group of experiments, $2 \cdot 10^4$ cells in 100 μl were exposed to 100 ng of [^{3}H]T-2 in 50 μl of solvent for 2 h and 6 h at 37° C. Following the incubation, the trays were centrifuged at 1500 rpm for 5 min, the supernatant was removed and any cells bound to the surface of the well were detached using 200 μl EDTA buffer. The cells were harvested and assayed as described in the first binding experiment.

Results

The primary mode of action of T-2 toxin on eukaryotic cells is the inhibition of protein synthesis [19]. As shown in Fig. 1, protein synthesis in the bovine MDBK cell line was inhibited by much lower toxin doses than were the CHO cells. Diminished protein synthesis was observed at 0.66 ng/ml and declined further to background levels by 5 ng/ml. Protein synthesis in CHO cells, how-

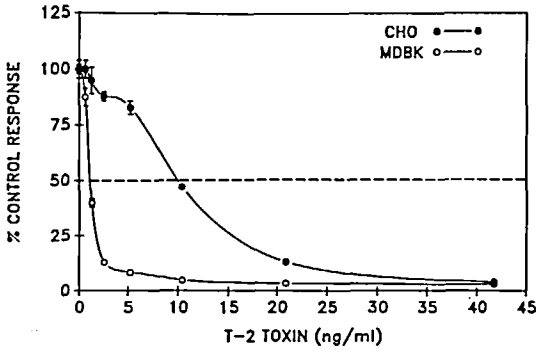

Fig. 1. Effect of T-2 toxin on protein synthesis. 100 μl of $2 \cdot 10^5$ stock CHO and MDBK cells/ml were incubated with 50 μl of T-2 toxin for 48 h. Following incubation, the cells were pulsed for 4 h with [^{3}H]Leu and assayed for protein synthesis. Results were converted to %control response ± S.E. and plotted as a function of toxin dose. Values lacking an error bar have standard errors too small to be plotted by the graphics program.

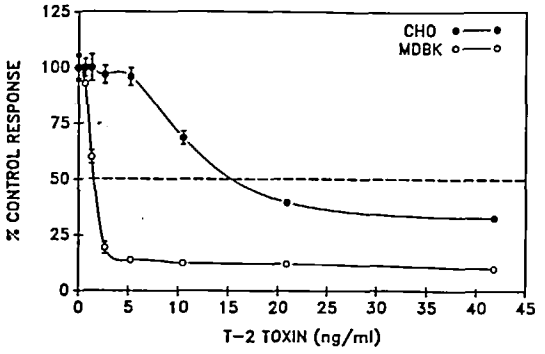

Fig. 2. Effect of T-2 toxin on mitochondrial activity. 100 μl of $2 \cdot 10^5$ stock CHO and MDBK cells/ml were incubated with 50 μl of T-2 toxin for 48 h. Following incubation, the cells were pulsed with MTT for 4 h and assayed for MTT reduction. Results were converted to %control response ± S.E. and were plotted as a function of toxin dose. Values lacking an error bar have standard errors too small to be plotted by the graphics program.

ever, exhibited little sensitivity to T-2 toxin until 2.6 ng/ml and the response gradually declined until minimal protein synthesis was observed at 35 ng/ml. The $TCID_{50}$ was 1 ng/ml and 10 ng/ml for MDBK and CHO, respectively.

The conversion by the cell of the tetrazolium salt MTT, into a colored formazan dye can be used as an indicator of cellular mitochondrial activity [20,21]. The effect of T-2 toxin on tetrazolium modification is shown in Fig. 2 and the dose response for this function paralleled that observed for protein synthesis in Fig. 1. Slightly diminished mitochondrial activity was first observed at 0.66 ng/ml for the MDBK and maximum inhibition was detected at 5 ng/ml. Effects on the CHO cell mitochondrial activity toward MTT were observed at much higher doses with initial inhibition beginning between 5 and 10

TABLE I

EFFECT OF 4 H T-2 MYCOTOXIN EXPOSURE ON CHROMIUM RELEASE BY CHO AND MDBK CELLS

CHO and MDBK cells were labeled with ^{51}Cr, washed as specified in the text and resuspended to a stock concentration of $20 \cdot 10^4$ cells/ml. 100 μl of the stock plus 50 μl of a 2 μg/ml T-2 stock or DME were added to quadruplicate wells of a 96-well tissue culture tray and incubated at 4° C or 37° C for 4 h or 24 h. The trays were centrifuged at 1500 rpm for 10 min and 50 μl of the supernatant was assayed for ^{51}Cr using a gamma counter. % of total release was calculated by dividing the experimental release by the total release value from the Triton X-100 control (total 4 h release at 4° C: CHO = 962 cpm, MDBK = 2087 cpm; 37° C: CHO = 1250 cpm, MDBK = 2122 cpm. Total 24 h release at 4° C: CHO = 1057 cpm, MDBK = 2068 cpm; 37° C: CHO-1169 cpm, MDBK = 2176 cpm).

Exposure time	Temperature	%Chromium release [a]			
		CHO		MDBK	
		DME	T-2	DME	T-2
4 Hour	4° C	10.0 ± 0.4	8.7 ± 0.5	1.4 ± 0.2	1.0 ± 0.2
	37° C	15.7 ± 0.8	16.2 ± 0.8	5.7 ± 0.2	11.3 ± 0.3 [a]
24 Hour	4° C	24.5 ± 0.9	12.2 ± 0.5 [a]	9.3 ± 0.7	9.6 ± 0.5
	37° C	42.2 ± 2.5	53.8 ± 3.3	24.7 ± 0.7	82.6 ± 0.5 [a]

[a] Significantly different from DME control, $P < 0.01$, as determined by Student's t-test.

ng/ml and maximum inhibition occurring at 20 ng/ml. The $TCID_{50}$ was 1.5 ng/ml and 15 ng/ml for MDBK and CHO, respectively.

T-2 toxin hemolyzes erythrocytes [12–16]. To examine the lytic effect of T-2 toxin on CHO and MDBK cells, the cell lines were labeled with ^{51}Cr and then incubated at 4°C with the toxin. The cells become metabolically quiescent at this temperature and, therefore, any release of ^{51}Cr from the cells should be a reflection of lytic effects rather than cell death. 4-h exposure to T-2 toxin at 4°C had little effect on the release of ^{51}Cr by either cell line (Table I) while a 4 h exposure at 37°C of these cells to T-2 toxin did have a significant effect on the MDBK cells ($P < 0.01$), doubling the amount of ^{51}Cr released from 5.7% to 11.3%. The CHO cells were relatively unaffected. MDBK cells incubated with T-2 toxin for 24 h at 4°C exhibited little effect, but this same exposure decreased the release of ^{51}Cr by the CHO cells by 50% (Table I). Both CHO and MDBK cells released significantly more ^{51}Cr after a 24-h treatment with T-2 toxin at 37°C with the MDBK releasing three times the amount of the medium control.

The susceptibility of a cell to the adverse effects of an agent may be a reflection of its ability to bind the toxicant. To examine this possibility, CHO and MDBK cell cultures were placed in ice water baths to reduce their metabolic activity and

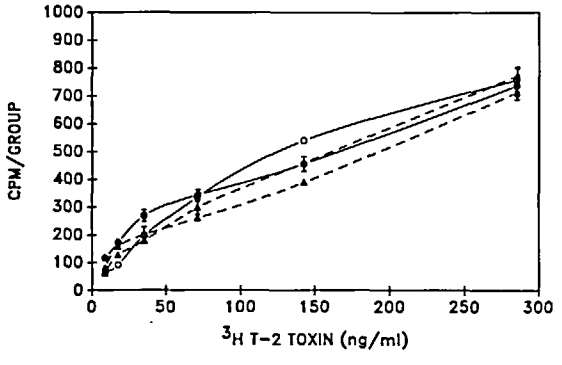

Fig. 3. Binding of [^{3}H]T-2 to cells. 100 µl of $2 \cdot 10^5$ stock CHO and MDBK cells/ml were incubated with varying concentrations of [^{3}H]T-2 in the presence (open symbols) or absence (filled symbols) of 625 ng of unlabeled T-2 toxin. The cells were harvested and assayed for bound [^{3}H]T-2 by liquid scintillation spectrometry. Solid line, MDBK; dashed line, CHO.

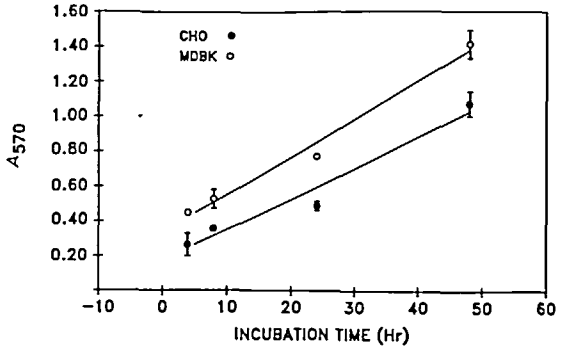

Fig. 4. Mitochondrial activity of CHO and MDBK cells. 100 µl of $2 \cdot 10^5$ stock CHO and MDBK cells/ml were incubated at 37°C and assayed for MTT reduction at 4 h, 8 h, 24 h or 48 h following the start of the experiment.

varying doses of radiolabeled T-2 were added to separate cell cultures. Following 5 h incubation at 4°C, the cells were assayed for bound [^{3}H]T-2 toxin and the results are shown in Fig. 3. Little difference in [^{3}H]T-2 binding between the two cell lines could be detected and the [^{3}H]T-2 toxin–cell interaction could not be blocked by the addition of excess unlabeled toxin. A plateau in binding was not readily observed for either cell line.

To determine whether the CHO and MDBK cells exhibit any differences in metabolism, the cells were assayed for mitochondrial activity, as indicated by MTT modification, at various periods over a 48-h timespan. The MDBK cells exhibited a higher mitochondrial activity than did the CHO cells (Fig. 4) at all time periods examined.

The increased mitochondrial activity toward MTT of the MDBK line could result in increased uptake of toxin by these cells. MDBK cells incubated at 37°C with [^{3}H]T-2 took up more of the labeled toxin than did the CHO cells. This difference was observed at both a 2 h (201 ± 12 cpm vs. 122 ± 1 cpm) and 6 h (172 ± 7 cpm vs. 81 ± 6 cpm) time periods for MDBK and CHO cells, respectively (data not shown).

Discussion

A previous study which examined the effect of T-2 toxin on various cultured cell lines failed to find any substantial differences in toxin activity on the cell lines tested [17]. Using an assay which

colorimetrically measures cellular mitochondrial activity [9,20,21], we expanded the study to examine toxin effects on cells from a wider variety of animal species and were able to find some cell lines that differed in toxin susceptibility [10]. The CHO cell line was found to be less susceptible to T-2 intoxication than the bovine cell line MDBK. These two cell lines were, therefore, used in the present study to examine the basis for this difference.

The primary effect of T-2 intoxication in the cell is the inhibition of protein synthesis [19]. A comparison of the effects of T-2 toxin on this function in our two cell lines showed that protein synthesis inhibition was observed at much higher doses in the CHO cell line than in the MDBK (Fig. 1) cells. The $TCID_{50}$ of 10 ng/ml for CHO protein synthesis, similar to previous reported values [17], is 10-fold higher than that found for the bovine cells. At least for protein synthesis, CHO cells are less sensitive to T-2 effects than MDBK cells.

Though protein synthesis is the primary site of T-2 action, many other functions can also be affected [3,5,7,8,10]. An examination of toxin effects on mitochondrial activity toward MTT reduction was performed in this study and the effects were found to parallel those of protein synthesis (Fig. 2). Mitochondrial inhibition in MDBK cells occurred at much lower doses than CHO cells and the $TCID_{50}$ was 10-fold lower for the bovine cells than the hamster cells. The inhibitory doses matched those of protein synthesis closely. T-2 toxin had previously been shown to exert inhibitory effects in mitochondria in yeast [7,8] and in isolated rat liver mitochondria [6]. It is not known whether the effects we observed in the present study reflect direct T-2 toxin effects on the cell mitochondria or are a consequence of the inhibition of cellular protein synthesis.

T-2 toxin can also mediate cellular effects at the membrane level. Previous studies have shown that T-2 toxin causes erythrocyte lysis indicating that the toxin interacts directly with the membrane [12–16]. The interaction of the toxin with red blood cells resulted in alterations in cell volume and shape which suggested that the toxin directly partitioned itself into the cell membrane [14,15]. To examine the lytic effects of T-2 toxin on the MDBK and CHO cell lines, the cells were labeled with radioactive chromium-51 and incubated at 4°C with the toxin. This temperature was used since the cells have diminished metabolic activity and endocytic uptake is minimal [22]. Any release of ^{51}Cr should, therefore, be the result of toxin–membrane interactions. The results summarized by Table I showed that no increase in release of ^{51}Cr occurred at 4 h or 24 h indicating that little cell lysis was induced. Interestingly, however, CHO cells exposed to T-2 toxin at 4°C for 24 h showed a decrease release of ^{51}Cr as compared to the controls (Table I). These results indicate that some cell–toxin interaction occurred that resulted in membrane protection. Similar protective effects by T-2 toxin against lysis were observed by Gyongyossy-Issa et al. for red blood cells [15]. Both cell lines exposed to toxin at 37°C released significantly more ^{51}Cr than controls, a reflection of cell death. MDBK cells released substantially more ^{51}Cr than CHO which further shows the increased sensitivity of the bovine cells to intoxication.

The activity of a particular agent can vary between animal species. The exotoxins of *Pseudomonas aeruginosa* and *Corynebacterium diphtheriae* both kill mammalian cells via exactly the same mechanism. The lethality of diphtheria toxin for murine cells, however, is 1000-fold less than the *Pseudomonas* exotoxin. This lowered toxicity is attributed to a lack of receptors for diphtheria toxin [23], which results in a decreased binding, internalization and cell death [24]. Other agents show similar species activity differences [25,26]. The differences in T-2 susceptibility in the current study could be a reflection of lowered toxin binding by CHO cells as compared to MDBK. The results of Fig. 3, however, show that both cell lines equally bind [^{3}H]T-2 toxin and the presence of excess unlabeled toxin has little apparent effect on the binding. At concentrations greater than 200 $TCID_{50}$ for MDBK, the binding to the cell did not plateau. These results are counter to those of previous reports [27] which showed that [^{3}H]T-2 binding was saturable and could be blocked by unlabeled T-2 toxin. These discrepancies may be a reflection of a difference in cell type (primary lymphocyte cultures vs. cultured cell lines) and also culture conditions.

Administration of T-2 toxin to animals results in damage to target tissues that have a component of respirationally active, rapidly dividing cells such as lymphoid tissues and the gut [28–30]. This characteristic appears to play a role in the altered sensitivity to T-2 intoxication observed in the current study. While both cell lines have similar doubling times (ATCC 1985 catalog, pp. 15, 34), the respiratory chain activity of the cells, as determined by mitochondrial MTT reduction, differed substantially. The MDBK cells were consistently more active toward the MTT substrate than the CHO cells, an indication of higher respiratory chain activity. Since both cell lines are epithelial in origin and previous studies have shown that cells of similar origin and function usually have similar numbers and kinds of mitochondria [31], the enzymatic activity required to convert MTT to a colored product is representative of mitochondrial function. The higher respiratory activity of MDBK cells could result in more T-2 toxin being taken up during cell growth and such an effect can be seen where MDBK and CHO cells were exposed at 37 °C to [^{3}H]T-2 toxin for varying times. At this temperature, which is optimum for cell growth, more [^{3}H]T-2 toxin was associated with MDBK cells than the CHO cells (data not shown), while at 4 °C both cells exhibited similar [^{3}H]T-2 association characteristics (Fig. 3).

T-2 toxin can be modified into a number of metabolic by-products by cellular esterase enzymes. This activity is especially pronounced in liver cells [32], but established tissue culture cell lines can also modify the toxin to a certain extent [33]. Differences in activity were observed between cell lines with CHO and a monkey cell line producing the most metabolites [32]. The susceptibility differences between CHO and MDBK may be a partial reflection of the ability of CHO cells to alter metabolically the T-2 toxin.

In conclusion, we have described two cell lines which differ in their susceptibility to T-2 intoxication. CHO cells are more resistant to T-2 toxin than MDBK cells and this is observed for protein synthesis and mitochondrial activity toward MTT. T-2 toxin does not have a detectable lytic effect on either cell line, but some membrane interaction does appear to occur. In cells which are respirationally diminished, an equal binding of radiolabeled toxin to both cell lines is observed which is not blocked by excess unlabeled toxin. When the two cell lines are cultured under optimum growth conditions, MDBK cell respiration is higher than CHO cells and more T-2 toxin is associated with these bovine cells. The greater sensitivity of the MDBK cells to T-2 toxin may be related to this increased uptake.

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