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Toxicity of the Mycotoxin Patulin for Rat Alveolar Macrophages[1]

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Agricultural workers are exposed to a variety of organic dusts containing fungi and their secondary metabolites. Patulin, a polyketide lactone mycotoxin produced by several common species of *Aspergillus* and *Penicillium*, is found in corn silage. Patulin is toxic in experimental animals and has been reported to be mutagenic, teratogenic, and carcinogenic. The cytotoxicity of patulin was studied in rat alveolar macrophages *in vitro*. The effects of patulin on membrane integrity were studied by measuring cell volume changes and release of ^{51}Cr. There was a significant release of ^{51}Cr after 1 hr exposure to submillimolar concentrations of patulin. Similarly, there was a significant decrease in ATP in cell cultures exposed to 0.5 mM patulin for 15 min and in cultures exposed to 0.05 mM patulin for 2 hr. There was a significant increase in mean cell volume after 2 hr exposure to 1.0 mM patulin but not after a 1 hr exposure. The effects of patulin on protein and RNA synthesis were studied by monitoring the incorporation of [^{3}H]leucine and [^{3}H]uridine, respectively. Inhibitions of protein and of RNA synthesis were both dose and time dependent. Protein synthesis was the most sensitive cellular parameter studied, with 50% inhibition after 1 hr at ca. 0.002 mM patulin. The data demonstrate that patulin is cytotoxic for rat alveolar macrophages *in vitro*.

Patulin, a polyketide lactone mycotoxin produced by several common species of *Aspergillus* and *Penicillium*, has been reported to be common in moldy corn silage (Escoula, 1974) and in naturally rotted apples (Stott and Bullerman, 1975). The initial interest in patulin was for its antimicrobial properties, but subsequent work has shown it to be toxic in experimental animals (Broom *et al.*, 1944; Ciegler *et al.*, 1971), carcinogenic in rats (Dickens and Jones, 1961), mutagenic in yeast (Mayer and Legator, 1969), and teratogenic in chick embryos (Ciegler *et al.*, 1976). Patulin was also shown to be the etiologic agent in an accidental epidemic of feed poisoning in Japan resulting in the mass death of 118 dairy cows in 1952 (Kurata, 1978). Patulin induces single- and double-strand breaks in HeLa DNA (Umeda *et al.*, 1972) and causes a high percentage of polyploid cells in human leukocyte cultures (Sentein, 1955). More recent studies have demonstrated that patulin (1) inhibits transcription in RNA synthesis in a cell-free system from rat liver (Moulé and Hatey, 1977), (2) inhibits translation of protein synthesis in rabbit reticulocyte lysates (Hatey and Gaye, 1978), and (3) inhibits syntheses of rRNA, tRNA, and probably mRNA in *Saccharomyces cerevisiae* (Sumbu *et al.*, 1983).

[1] Mention of trade names is for information only and does not constitute endorsement over other products not mentioned.

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The purpose of this investigation was to study the toxicity of patulin in rat alveolar macrophages *in vitro*. Pulmonary macrophages perform several important functions in the lung, including phagocytosis of living and nonliving foreign particles, regulation of T-lymphocyte proliferation, provision of T-helper activity for antibody production, and production of mediators of cellular immunity (Lipscomb *et al.*, 1981). Thus, cytotoxic damage to alveolar macrophages could lead to serious pulmonary and/or systemic damage.

MATERIALS AND METHODS

Mycotoxin

Patulin was obtained from Aldrich Chemical Company (Milwaukee, Wisc.) and fresh stock solutions were prepared daily for each experiment. In all experiments except ^{51}Cr release, dimethyl sulfoxide (DMSO) was used as the solvent for patulin and the final concentrations of DMSO and $NaHCO_3$ were 2.0% and 15mM, respectively. Because of the restraints of the experimental system and a desire to have a lower final DMSO concentration, a mixture of 5% DMSO, 5% dimethylformamide (DMF), and 175 mM $NaHCO_3$ was used as the solvent for patulin in the ^{51}Cr-release experiments. In this case, the final concentrations of the solvent components were 0.5% DMSO, 0.5% DMF, and 25mM $NaHCO_3$, respectively. Appropriate solvent controls were included in all experiments.

Alveolar Macrophage Isolation and Culture

Alveolar macrophages (AM) were harvested from male Long–Evans hooded rats by tracheal lavage according to the method of Myrvik *et al.*, (1961). Rats were anesthetized by intraperitoneal injection with sodium pentobarbital and exsanguinated by cutting the abdominal aorta. The lungs from each rat were lavaged with a total of 60 ml of prewarmed calcium- and magnesium-free Hanks' balanced salt solution (HBSS). The cells from several animals were pooled, centrifuged at 500*g* for 10 min, 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, N.Y.). A Coulter counter (Coulter Instrument Co., Hialeah, Fla.) was employed to determine the cell concentration. Cell purity, as determined from the cell volume distribution, was approximately 95%. Somerville *et al.*, (1984) have shown that such a distribution count includes all subpopulations of alveolar macrophages obtained by pulmonary lavage.

AM suspensions were cultured at a cell concentration of 2.5×10^5 cells/cm^2 in Linbro tissue culture plates (Linbro, Hamden, Colo.). AM monolayers, in Medium 199 (2% HI-FBS, H, P, S), were incubated for 2 hr at 37°C in 5% CO_2 to allow for adherence of AM, rinsed with PBS to remove nonadherent cells, and incubated with Medium 199 (10% HI-FBS, P, S) until needed for experiments. The viability of the cultured AM was routinely greater than 95% as determined by trypan blue exclusion (Phillips, 1973).

Determination of Mean Cell Volume

Mean cell volume (MCV) was determined in treated and control cells using a Coulter Model ZB electronic cell counter (Coulter Instrument Co.) equipped with a Coulter Channelyzer attachment. The following equation (Castranova *et al.*, 1979) was employed to determine the MCV values:

$$\text{MCV} = \text{corrected channel no.} \times \text{calibration factor.}$$

The calibration factor was determined by sizing particles of known diameter (Paper Mullberry Pollen: diam = 13 μm; Coulter Instrument Co.).

The alveolar macrophages were suspended in Hanks' balanced salt solution with and without 5 mM glucose as an exogenous energy source. The cells were maintained in 17 × 100-mm culture tubes on a shaking water bath incubator at 37°C. Treatments were initiated at 10-min intervals to compensate for the time required to determine MCV for each sample (ca. 6 min). Alveolar macrophages tend to swell during isolation and maintenance at low temperature and then shrink to normal size at 37°C. In order to eliminate any bias due to cell volume changes during preincubation before treatment, the experiment was done in a randomized complete block experimental design (i.e., the four treatments were randomized within each of four sets of replications). The 1- and 2-hr exposure experiments were repeated on three separate days each.

Chromium Release Assay

Freshly isolated AM at a cell concentration of 1.0×10^7 cells/ml were incubated with ^{51}Cr-labeled sodium chromate (New England Nuclear Corp., Boston, Mass.) at a ratio of 50 μCi/10^7 cells in Medium 199 (10% HI-FBS, P, S) for 45 min at 37°C in 5% CO_2. The specific activity of the sodium chromate was 296.3 μCi/mg. Labeled AM were washed three times with HBSS and resuspended in Medium 199 at a cell concentration of 2.5×10^6 cells/ml. Next, 0.1 ml of the labeled cell suspension was added to a series of test tubes along with 0.1 ml of Medium 199 containing the appropriate concentration of patulin or medium only. Control and treated AM were incubated for ≤2 hr at 37°C in 5% CO_2. After incubation, the cells were pelleted by centrifugation at 550*g* and 0.1 ml of the supernatant was transferred to a clean tube and counted in a gamma scintillation counter. Total release of chromium from AM was obtained by lysing the cells with 0.1% Triton X-100. The percentage chromium release equals the experimental release value divided by the total release value. Both values were corrected for spontaneous release.

Adenosine Triphosphate Determination

ATP levels in treated and untreated monolayer cultures were determined by the luciferin–luciferase assay. At the time of treatment, the medium was removed by aspiration and replaced with fresh medium containing patulin. Sodium iodoacetate was used as a positive control. After treatment, the medium was removed by aspiration and 200 μl of releasing reagent (Diagnostic Sciences, Inc.,

San Diego, Calif.) was added to each well. After a 45-sec extraction time, the releasing reagent was diluted 20-fold and the sample put in crushed ice. Buffered firefly lantern extract (Sigma FLE-50; Sigma Chemical Co., St. Louis, Mo.) was rehydrated, stored overnight at 4°C, and used as a source of luciferin–luciferase. ATP determinations were done in a darkened laboratory with an ATP photometer (SAI Technology Co., San Diego, Calif.) using dark-adapted scintillation vials to avoid interference due to chemiluminescence. Quantitation was done by the method of internal standardization.

Macromolecular Synthesis

For protein-synthesis studies, AM monolayers were incubated in Eagle's minimum essential medium with Earle's salt solution and L-glutamine but without L-leucine (KC Biological). The AM monolayers were incubated with patulin and 1 μCi/ml [^{3}H]leucine (110 Ci/mmole; New England Nuclear Corp.) at 37°C in 5% CO_2 for up to 4 hr. After the desired incubation interval the culture plates were chilled on ice to stop metabolism, the culture medium was removed, and the cells were washed three times with chilled PBS. Monolayers were solubilized for 30 min with 0.5 ml of 0.1 M KOH and proteins were acid precipitated with 2.0 ml of 10% trichloroacetic acid (TCA) and 50 μl of 1% bovine serum albumin (BSA). The acid-precipitable material was collected on glass-fiber filters, washed with cold TCA, and placed in scintillation vials with 10 ml Aquasol-2 (New England Nuclear Corp.) and counted by liquid scintillation.

For RNA-synthesis studies the treated and untreated AM cultures were incubated with 2 μCi/ml [^{3}H]uridine (29.3 Ci/mmole; New England Nuclear Corp.). After the desired incubation time the cultures were chilled on ice and rinsed with cold PBS. The monolayers were solubilized for 30 min with 0.25 ml 0.1 M KOH and the solubilized suspension was added to 10 ml Aquasol-2 and counted by liquid scintillation.

Statistical Analysis

The data from the mean cell volume experiments were analyzed by two-way analysis of variance (ANOVA) on the West Virginia University mainframe computer using the Statistical Analysis System (SAS) software. Data from the other experiments were analyzed by comparing individual treatments with the untreated controls by the Student t test. A one-tail t test was used for ^{51}Cr release and ATP experiments ($P < 0.05$) because we were concerned only with the toxic effects of patulin. A two-tail t test was used for protein and RNA synthesis experiments ($P < 0.05$) to allow for the possibility of stimulation as well as inhibition.

RESULTS

Alveolar macrophages exposed to patulin in Hanks' solution for 2 hr showed a significant increase ($P < 0.0001$) in mean cell volume (Tables 1 and 2) whereas there was no increase in MCV after a 1-hr exposure interval (data not shown). Analyses of variance demonstrated that the presence or absence of 5 mM glucose in the medium had no effect on MCV ($P > 0.05$).

TABLE 1
CELL VOLUME CHANGES FOLLOWING EXPOSURE OF ALVEOLAR MACROPHAGES TO PATULIN FOR 2 hr

Treatment	Patulin[a]	Glucose[b]	MCV[c]	CV (%)[d]
A	−	+	1226.7 ± 91.0	7.4
B	+	+	1372.4 ± 82.3	6.0
C	−	−	1226.6 ± 95.1	7.8
D	+	−	1377.3 ± 83.6	6.1

[a] Concentration = 1.0 mM.
[b] Concentration = 5.0 mM.
[c] Mean cell volume (mean ± standard deviation).
[d] Coefficient of variation.

Release of ^{51}Cr from alveolar macrophages following exposure to patulin was both time and concentration dependent (Fig. 1). Treatment of these cells at ≥0.15 mM patulin caused significant leakage of ^{51}Cr within 30 min (Table 3).

Adenosine triphosphate concentrations in AM monolayer cultures were markedly inhibited within 1 hr at concentrations of ≥0.05 mM patulin (Table 4). ATP experiments were done with both the DMSO and DMSO/DMF/$NaHCO_3$ solvents for comparison. The results were nearly identical (data not shown) suggesting that the cells can tolerate up to 2% DMSO or 25 mM $NaHCO_3$ without ill effect.

Incorporation of ^3H-labeled precursors into protein and RNA was strongly inhibited by patulin (Table 5). Inhibition was both time and concentration dependent for both classes of macromolecules but protein synthesis was sensitive to 10 to 100-fold lower concentrations of patulin at the same time interval. For example protein synthesis was significantly inhibited within 30 min at 0.001 mM patulin whereas RNA synthesis was inhibited to a lesser extent at the same exposure time at 0.1 mM patulin. The dose producing 50% of inhibition at 1 hr (ED_{50}) was estimated at ca. 0.0016 and 0.019 mM for [^3H]leucine and [^3H]uridine incorporation, i.e., for protein and RNA synthesis, respectively. Although 0.1 mM patulin significantly inhibited RNA synthesis, there was a consistent and significant ($P < 0.05$) increase in [^3H]uridine incorporation at 0.01 mM patulin.

TABLE 2
ANALYSIS OF VARIANCE OF CELL VOLUME CHANGES FOLLOWING 2-hr EXPOSURE OF ALVEOLAR MACROPHAGES TO PATULIN

Source	Degrees of freedom	F Value	$P > F^a$
Glucose (G)	1	0.01	0.9254
Patulin (P)	1	33.89	0.0001
G × P interaction	1	0.01	0.9221

[a] The probability of obtaining an F value greater than the value observed.

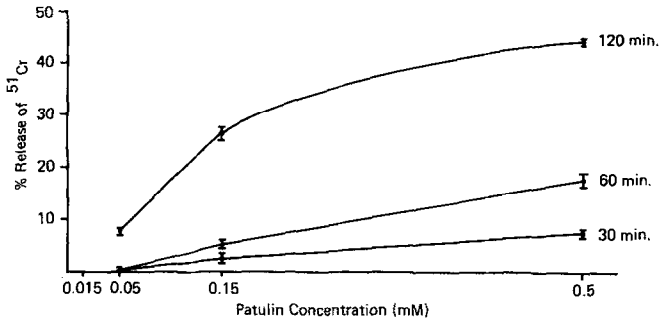

FIG. 1. Effect of patulin concentration and incubation time on ^{51}Cr release in rat alveolar macrophages.

DISCUSSION

Patulin is toxic to rat alveolar macrophages *in vitro,* causing an increase in mean cell volume, leakage of ^{51}Cr from the cell, a decrease in cellular ATP, and inhibition of protein and RNA synthesis (Tables 1–5).

Mean cell volume and ^{51}Cr release reflect membrane transport properties. The

TABLE 3

^{51}Cr RELEASE FOLLOWING SHORT-TERM EXPOSURE OF ALVEOLAR MACROPHAGES TO PATULIN

Treatment	cpm[a]	Probability[b]	% Release[c]
30-min exposure			
Spontaneous release[d]	1,766.4 ± 52.7		
Total release[e]	12,018.0 ± 163.5		
0.05 mM patulin	1,811.6 ± 24.3	>0.05	0.4
0.15 mM patulin	2,048.2 ± 87.0	<0.0002	2.7
0.5 mM patulin	2,562.8 ± 57.3	<0.0001	7.8
60-min exposure			
Spontaneous release	2,216.0 ± 60.2		
Total release	12,874.8 ± 453.6		
0.05 mM patulin	2,273.4 ± 14.2	<0.05	0.5
0.15 mM patulin	2,763.2 ± 101.0	<0.0001	5.1
0.5 mM patulin	4,092.4 ± 130.7	<0.0001	17.6
120-min exposure			
Spontaneous release	2,330.6 ± 116.0		
Total release	12,319.4 ± 129.9		
0.015 mM patulin	2,156.4 ± 103.4	>0.05	−1.7
0.05 mM patulin	3,074.4 ± 76.7	<0.0001	7.4
0.15 mM patulin	4,967.6 ± 133.1	<0.0001	26.4
0.5 mM patulin	6,754.4 ± 57.3	<0.0001	44.3

[a] Counts per minute; average of 5 replicate tubes ± standard deviation.

[b] One-tailed *t* test, 8 degrees of freedom.

[c] % release = (treated-spontaneous)/(total-spontaneous).

[d] Untreated control cells.

[e] Cells lysed with Triton X-100.

TABLE 4
EFFECT OF PATULIN ON ATP LEVELS IN RAT ALVEOLAR MACROPHAGE CULTURES[a]

Concentration (mM)	ATP (ng/ml)	Percentage of control
Medium control[b]	287.8 ± 26.2	100.0
Solvent control[c]	323.5 ± 69.6	112.4
0.0015	274.9 ± 43.2	95.5
0.005	253.7 ± 34.9	88.2
0.015	216.5 ± 21.7	75.2
0.05	180.2 ± 38.7[e]	62.6
0.15	99.6 ± 10.5[e]	34.6
0.5	33.9 ± 5.3[e]	11.8
Positive control[d]	67.6 ± 12.1[e]	23.5

[a] 1-hr exposure at 37°C in 5% CO_2.
[b] 20 μl of medium used in place of DMSO.
[c] 20 μl of DMSO; final concentration = 2.0% DMSO.
[d] 0.3 mM sodium iodoacetate.
[e] Significant, one-tail t test, $P < 0.05$.

Na^+–K^+ pump (Na^+–K^+ ATPase) maintains cellular volume by transporting Na^+ out of the cell against a concentration gradient. Therefore, steady state (i.e., constant mean cell volume) depends on a balance between inward leakage of Na^+ and the active outflux of Na^+ by the pump. ATP is the energy source for the

TABLE 5
INHIBITION OF INCORPORATION OF [^{3}H]LEUCINE AND [^{3}H]URIDINE IN ALVEOLAR MACROPHAGE CULTURES BY PATULIN

	[^{3}H]Leucine[a]			[^{3}H]Uridine[b]		
Exposure (hr)	Concentration (mM)	cpm[c] ($\bar{X}$ ± SD)	% Control	Concentration (mM)	cpm[c] ($\bar{X}$ ± SD)	% Control
0.5	Control	262.3 ± 37.8	100.0	Control	2445.2 ± 893.7	100.0
	0.001	186.7 ± 30.7[d]	71.2	0.01	2522.5 ± 443.7	103.2
	0.01	54.7 ± 12.4[d]	20.8	0.1	1686.6 ± 318.3[d]	69.0
	0.1	39.6 ± 10.5[d]	15.1	1.0	537.4 ± 470.0[d]	22.0
1.0	Control	583.4 ± 100.2	100.0	Control	3454.6 ± 639.6	100.0
	0.001	475.3 ± 74.1[d]	81.5	0.01	3989.6 ± 607.0	115.5
	0.01	77.5 ± 9.6[d]	13.3	0.1	1719.2 ± 462.4[d]	49.8
	0.1	38.1 ± 10.9[d]	6.5	1.0	219.3 ± 90.0[d]	6.3
2.0	Control	1634.2 ± 497.4	100.0	Control	5315.8 ± 1469.8	100.0
	0.001	969.3 ± 368.4[d]	59.3	0.01	6792.3 ± 1669.0[d]	127.8
	0.01	125.7 ± 40.3[d]	7.7	0.1	2211.3 ± 1134.9[d]	41.6
	0.1	51.2 ± 9.4[d]	3.1	1.0	184.3 ± 53.5[d]	3.5
4.0	Control	2262.1 ± 645.4	100.0	Control	8004.8 ± 1649.3	100.0
	0.001	1203.6 ± 269.7[d]	53.2	0.01	15318.9 ± 2963.9[d]	191.4
	0.01	114.6 ± 23.9[d]	5.1	0.1	710.4 ± 455.5[d]	8.9
	0.1	45.0 ± 9.2[d]	2.0	1.0	222.0 ± 103.3[d]	2.8

[a] Used to monitor protein synthesis.
[b] Used to monitor RNA synthesis.
[c] Counts per minute (average ± standard deviation); all values are based on two experiments with four replications each.
[d] Significantly different from the control, two-tail t test, $P < 0.05$.

active outflux of Na^+ by the pump (Tosteson and Hoffman, 1960). After 1 hr of treatment with relatively low concentrations of patulin (0.05 mM), there was evidence of leakage of ^{51}Cr and a decrease in cellular ATP while cell volume was maintained. These results suggest that the Na^+–K^+ pump was able to compensate for this leakage in spite of reduced ATP levels in the cells. The decrease in ATP could be due to (1) a direct effect on energy metabolism, (2) increased use of ATP by the Na^+–K^+ ATPase system, or both. After 2 hr, there was a significant increase in mean cell volume resulting from leakage, decrease in ATP, and/or direct inhibition of Na^+–K^+ ATPase. Singh (1967) reported that patulin has a direct effect on aerobic respiration; Phillips and Hayes (1979) reported that patulin inhibited Na^+–K^+ ATPase activity within 5 min at a relatively high patulin concentration (3 mM) and within 30 min at 0.5 mM patulin in cell-free preparations from toad bladder. On the basis of the results of Phillips and Hayes (1979), one might expect a more rapid increase in mean cell volume than was observed, but in several experiments, the mean cell volume of cells treated with 1.0 mM patulin was not significantly different from that of control cells ($P > 0.05$). Perhaps the Na^+–K^+ ATPase of rat pulmonary macrophages is somewhat less sensitive to patulin than the enzyme prepared from toad bladder. The comparison of sensitivity is difficult because intact cells were used in the present study. Furthermore, direct inhibition of Na^+–K^+ ATPase would not be expected to result in an immediate increase in mean cell volume. Phillips and Hayes (1979) also studied the effect of patulin on electrogenic Na^+ transport by means of the short-circuit current apparatus and demonstrated significant impairment of transport by patulin. The high linear correlation ($r = 0.996$) of their data suggested a causal relationship between Na^+–K^+ ATPase and electrogenic Na^+ transport. Ueno *et al.* (1976) reported that patulin impairs the Na^+-dependent glycine transport system of reticulocytes. Thus, it is clear that patulin is toxic to cell membrane function in several *in vitro* systems.

Among the various parameters reported here, inhibition of protein synthesis was the most remarkable in terms of both the rapidity of the response and the low concentration of patulin required. For example, protein synthesis after a 30-min treatment at 0.001 mM patulin was 71.2% of the untreated control ($P < 0.05$) whereas RNA synthesis was inhibited to 69.0% of control after 30 min treatment at 0.1 mM patulin ($P < 0.05$). Thus, there was a similar degree of inhibition within the same time interval, but a 100-fold higher concentration was required to inhibit RNA synthesis. Membrane damage, as expressed by release of ^{51}Cr from the cells, occurred within 30 min at 0.15 mM patulin, and cellular ATP levels in cells treated for 1 hr at 0.05 mM patulin were significantly lower than untreated control cells. Thus, RNA synthesis, ^{51}Cr release, and cellular ATP were roughly equivalent in their sensitivity to patulin. Although there was a highly significant difference ($P < 0.0001$) in mean cell volume after 2 hr exposure to 1.0 mM patulin, there was no difference after 1 hr. Thus the five parameters can be rank ordered in terms of sensitivity as follows: protein synthesis $>>$ RNA synthesis $\geq$ ^{51}Cr release $\simeq$ ATP level $>$ mean cell volume. These findings permit speculation as to the mode of action in alveolar macrophages. The data suggest that the primary toxic event in these cells involves inhibition of protein synthesis. A consistent enhancement of RNA synthesis was observed at 0.01 mM patulin ($P > 0.5$ at 1

hr but $P < 0.05$ after 2 and 4 hr). At this concentration, protein synthesis was ≤20% of control at all exposure intervals. If conditions for RNA synthesis are otherwise favorable, inhibition of protein synthesis could make relatively more ATP available. This might explain the moderate stimulation of RNA synthesis. Inhibition of RNA synthesis at higher patulin concentrations could result from direct inhibition of synthetic enzymes. Depletion of specific protein(s) required for RNA synthesis could also have a contributory effect. On the other hand, it is unlikely that inhibition of protein synthesis alone could cause the effects on RNA synthesis, ^{51}Cr release, mean cell volume, and ATP levels observed. For example, Gerberick *et al.* (1984) reported that T-2 toxin caused immediate cessation of protein synthesis in rat alveolar macrophage cultures at a 0.1 μM concentration. Therefore, T-2 toxin is approximately 2 orders of magnitude more toxic to protein synthesis in these cells than patulin, yet T-2 toxin had no measureable effect on mean cell volume, ^{51}Cr release, or ATP levels after 6 hr exposure at levels of T-2 toxin which strongly inhibited protein synthesis. T-2 toxin caused a decrease in mean cell volume after 18 hr exposure which appeared to be the result of cell disintegration. Thus the action of patulin in these cells is distinct from that of T-2 toxin.

A number of authors have ascribed the toxic activity of patulin to interaction with SH groups (Singh, 1967; Ueno *et al.*, 1976; Hatey and Gaye, 1978; Phillips and Hayes, 1979) and it has been reported that the inhibition of glycine transport in reticulocytes (Ueno *et al.*, 1976) and inhibition of protein synthesis (Hatey and Gaye, 1978) can be reduced or prevented by prior addition of dithiothreitol or glutathione. Blockage of SH groups by compounds other than patulin has been shown to increase K^+ and Na^+ leakage (Vincent and Blackburn, 1958; Sutherland *et al.*, 1967), to inhibit active Na^+ and K^+ transport (Rega *et al.*, 1967), and to inhibit Na^+–K^+ ATPase (Godin and Schier, 1972) in human erythrocytes.

Previous studies have demonstrated the inhibitory properties of patulin toward incorporation of labeled precursors into DNA (replication), RNA (transcription), and protein (translation). The relative sensitivity of these processes to patulin has shown some variation but *in vitro* translation may be more sensitive to patulin than *in vitro* transcription (Hatey and Gaye, 1978). The comparisons reported here were consistent and reproducible and in some experiments were made in side-by-side comparisons on the same day with a common cell population and common stock solutions.

Patulin was shown to inhibit critical cellular functions in cultured alveolar macrophages. Therefore, inhalation of airborne silage or grain dust particulates contaminated with patulin could have deleterious effects on normal macrophage function.

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