ENVIRONMENTAL RESEARCH 41, 505–513 (1986)

Toxicity of Penicillic Acid for Rat Alveolar Macrophages *in Vitro*

W. G. SORENSON*,† AND JANET SIMPSON*

*Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown, West Virginia 26505, and †Department of Microbiology, West Virginia University, Morgantown, West Virginia 26506

Received June 1985

Penicillic acid (PA) is a polyketide mycotoxin produced by several species of *Aspergillus* and *Penicillium*. This mycotoxin is toxic in experimental animals and has also been reported to be carcinogenic. The cytotoxicity of penicillic acid was studied in rat alveolar macrophages (AM) *in vitro*. The effects of penicillic acid on membrane integrity were studied by measuring cell volume changes and ^{51}Cr release. There was significant ^{51}Cr release after 2 hr exposure to 1.0 mM penicillic acid, but not after 1 hr exposure. There was a significant decrease in adenosine triphosphate (ATP) in cell cultures exposed to 1.0 mM penicillic acid for 4 hr. Inhibition of the incorporation of [^{3}H]leucine into protein was both dose- and time-dependent and protein synthesis was inhibited significantly after 2 hr exposure to $\geq$0.1 mM penicillic acid. RNA synthesis was inhibited to a lesser extent than protein synthesis. Although there was a significant inhibition of RNA synthesis at 1.0 mM PA after 4 hr, there was no inhibition of RNA synthesis even after 4 hr at any concentration <1.0 mM. The ED_{50} dose after 2 hr exposure was 0.18 and 0.60 mM for protein and RNA synthesis, respectively. There was significant inhibition of phagocytosis after 2 hr exposure at $\geq$0.3 mM penicillic acid and the ED_{50} for phagocytosis was 0.09 mM. Thus phagocytosis was more sensitive to the toxic effects of penicillic acid than any other cellular process studied. The results reported in this study are similar to those observed for patulin in an earlier study from our laboratory except that patulin was generally more toxic to alveolar macrophages than penicillic acid. The data demonstrate that penicillic acid is toxic to rat alveolar macrophages *in vitro* and suggest the possibility of a respiratory hazard to agricultural workers exposed to contaminated grain.

INTRODUCTION

Penicillic acid (PA) is a highly reactive, unsaturated lactone mycotoxin produced by several species of *Penicillium* and *Aspergillus* including some species known to produce other mycotoxins. The fungi which produce this mycotoxin are frequently isolated from common agricultural commodities and penicillic acid has been shown to be present in animal feed (Wilson, 1976), tobacco (Snow *et al.*, 1972), and blue-eye diseased corn (Kurtzman and Ciegler, 1970). Penicillic acid is structurally similar to patulin and shares many of the cytotoxic properties of patulin (Busby and Wogan, 1981). Murnaghan (1946) reported that penicillic acid has "digitalis-like" action on cardiac muscle, causes dilation of systemic blood vessels, and has antidiuretic properties. Penicillic acid is bacteriostatic, cytotoxic to mammalian cells producing inhibition of mitosis and chromosomal abnormalities (Umeda *et al.*, 1977; Wilson, 1976; Umeda, 1971; Natori *et al.*, 1970), carcinogenic in rats (Dickens and Jones, 1961, 1965), mutagenic in mouse mammary carcinoma cells (Umeda *et al.*, 1977), and it causes single- and double-strand breaks in HeLa and mammary carcinoma DNA (Umeda *et al.*, 1972). Macromo-

0013-9351/86 $3.00

lecular synthesis was inhibited by penicillic acid in HeLa cells (Kawasaki *et al.*, 1971) and *Tetrahymena pyriformis* (Hayes, 1977). Where dose comparisons have been made between penicillic acid and patulin, the latter mycotoxin was generally found to produce its toxic effects at a lower dose and to a greater degree than penicillic acid.

The purpose of this investigation was to study the toxicity of penicillic acid in rat alveolar macrophages *in vitro*. Pulmonary alveolar 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

Mycotoxins. Penicillic acid was obtained from Aldrich Chemical Company (Milwaukee, Wisc.) and fresh stock solutions were prepared daily in dimethyl sulfoxide (DMSO) for each experiment. Appropriate solvent controls were included in all experiments.

Alveolar macrophage isolation and culture. Alveolar macrophages were harvested from male Long–Evans hooded rats by tracheal lavage (Gerberick and Sorenson, 1983). In brief, the cells from several animals were pooled, washed by centrifugation at 550*g*, resuspended in medium 199 containing 2% fetal bovine serum (FBS) and antibiotics (100 u/ml penicillin G and 100 μg/ml streptomycin sulfate), and plated in Linbro tissue culture plates (Linbro, Hamden, Colo.). After a 2-hr incubation at 37°C in 5% CO_2 to allow adherence, the medium was replaced with fresh Medium 199 containing 10% FBS until needed for experiments. Unless indicated otherwise, cells were cultured in Medium 199 containing 10% FBS and were incubated at 37°C in a humidified atmosphere in 5% CO_2. Exposure times ranged from 0.5 to 4 hr for the various experiments; they are indicated in the tables. The cell preparations appeared to be homogeneous populations of alveolar macrophages based on phagocytosis and nonspecific esterase studies (Gerberick and Sorenson, 1983). The viability of the alveolar macrophage preparations were consistently 90–95% based on trypan blue dye exclusion.

Determination of mean cell volume. Mean cell volume was determined using a Coulter counter equipped with Channelyser following treatment in suspension in Hanks' balanced salt solution (Sorenson *et al.*, 1985).

Chromium release assay. Freshly isolated AM were incubated with sodium [^{51}Cr]chromate for 45 min, washed three times, and resuspended in Medium 199. Aliquots (0.1 ml) of labeled cells, along with 0.1 ml of Medium 199 containing the mycotoxin or solvent control, were incubated for the desired time. After incubation, 0.1 ml of supernatant (550*g*) was transferred to a clean tube and counted in a gamma scintillation counter. Total release was obtained by lysing the cells with 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 (Sorenson *et al.*, 1985).

Adenosine triphosphate (ATP) determination. ATP levels in treated and un-

treated monolayer cultures were determined by the luciferin–luciferase assay (Sorenson *et al.*, 1985). At the time of treatment, the medium was removed by aspiration and replaced with fresh medium containing mycotoxin. Sodium iodoacetate was used as a positive control. ATP was extracted directly without removing the cells from the plates. ATP determinations were done in a darkened laboratory with an ATP photometer using dark-adapted scintillation vials.

Macromolecular synthesis. For protein-synthesis studies AM monolayers were incubated in Minimum Essential Medium (Eagle) with L-glutamine but lacking leucine (Sorenson *et al.*, 1985). The cells were incubated with mycotoxin and [^{3}H]leucine (1 μCi/ml). After the desired incubation interval the culture plates were chilled on ice, the medium removed and the cells washed, and the monolayers solubilized. Proteins were precipitated in hot trichloroacetic acid (TCA) and TCA-precipitable material was collected on glass-fiber filters, washed, placed in scintillation vials, and counted by liquid scintillation (Sorenson *et al.*, 1985).

For RNA synthesis studies the treated and untreated AM cultures were incubated with [5-^{3}H]uridine (1 μCi/ml). After incubation, the cultures were chilled on ice, rinsed, and solubilized. The solubilized material was counted by liquid scintillation (Gerberick *et al.*, 1984).

Phagocytosis of ^{51}Cr-labeled sheep erythrocytes. AM monolayers were prepared in Linbro tissue culture plates and incubated with PA. After exposure, the medium was removed and 0.5 ml of fresh medium containing 2×10^7 ^{51}Cr-labeled sheep red blood cells was added (Snydermann *et al.*, 1977). The monolayers were then incubated 1 hr. After incubation the medium was removed and the bound extracellular sheep cells were lysed with lysing medium (Snydermann *et al.*, 1977). The monolayers were washed twice more with lysing medium, dissolved with 0.5% SDS, and counted by gamma scintillation (Snydermann *et al.*, 1977).

RESULTS

Penicillic acid caused a significant increase in mean cell volume after 2 hr exposure at 1.0 mM (Table 1). There was significant chromium release within 30 min exposure to a concentration of 0.1 mM PA. Chromium release was both dose- and

TABLE 1
CELL VOLUME CHANGES FOLLOWING 2 HR EXPOSURE OF ALVEOLAR MACROPHAGES TO PENICILLIC ACID

Treatment (mM)	MCV[a]
Control[b]	1238.8 ± 33.8
0.1	1167.5 ± 49.4
0.3	1151.9 ± 58.5
1.0	1332.5 ± 41.8[c]

[a] Mean cell volume in cubic micrometers; average of 8 replicate samples ± SD.
[b] Cells treated with the carrier solvent in the medium.
[c] Significantly larger than controls, one-tailed t test, $P < 0.05$.

time-dependent (Table 2) and reached a value of 11.9% after 2 hr exposure to 1.0 mM PA.

Penicillic acid had relatively little effect on ATP levels in these cells. In four separate experiments, 1.0 mM penicillic acid always produced a significant decrease ($P < 0.05$) in ATP levels compared to untreated controls after 4 hr exposure (Table 3). On the other hand, the results at 2 hr were less consistent and there was a significant difference in only three of the four experiments. The data shown in Table 3 are from the experiment in which the difference was insignificant. We chose to be conservative because the data are not unequivocal. As can be seen in Table 3, however, 2 hr exposure to 1.0 mM penicillic acid led to a moderate decrease in ATP. There was no significant decrease in ATP even after 4 hr at any concentration <0.3 mM.

Penicillic acid inhibited protein synthesis after 2 hr exposure at 0.3 mM (Table 4). Penicillic acid had less effect on RNA synthesis than on protein synthesis (Table 5). There was a significant inhibition of RNA synthesis after 2 and 4 hr exposure to 0.3 mM penicillic acid. On the other hand there was no inhibition of RNA synthesis at <0.3 mM even after 4 hr exposure.

Penicillic acid had a significant effect on phagocytosis (Table 6). It is noteworthy that phagocytosis was the most sensitive parameter studied and there was significant inhibition ($P < 0.05$) at 2 hr exposure at 0.03 mM penicillic acid. The

TABLE 2
EFFECT OF PENICILLIC ACID ON CHROMIUM RELEASE FROM ALVEOLAR MACROPHAGES IN CULTURE

Concentration (mM)	cpm[a]	% Release[b]
30 min exposure		
Control[c]	865.0 ± 20.1	
0.03	888.5 ± 35.5	0.3
0.1	918.7 ± 13.5[d]	0.7
0.3	926.0 ± 24.9[d]	0.8
1.0	1150.7 ± 45.3[d]	3.9
60 min exposure		
Control[c]	908.8 ± 18.3	
0.03	910.3 ± 34.6	0.0
0.1	931.5 ± 15.4[d]	0.4
0.3	975.0 ± 48.6[d]	1.2
1.0	1340.2 ± 43.7[d]	7.8
120 min exposure		
Control[c]	991.8 ± 38.2	
0.03	1002.8 ± 19.3	0.2
0.1	1044.0 ± 17.4[d]	0.9
0.3	1380.0 ± 114.7[d]	6.3
1.0	1721.3 ± 82.2[d]	11.9

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

[b] % Release = (treated-control)/(total-control); total release was determined by lysing the cells with Triton X-100.

[c] Cells treated with the carrier solvent in the medium.

[d] Significantly higher than the control; one-tailed t test, 10 df.

TABLE 3
EFFECT OF PENICILLIC ACID ON LEVELS OF ADENOSINE TRIPHOSPHATE IN ALVEOLAR MACROPHAGE CULTURES

Concentration (mM)	Exposure time (hr)		
	1	2	4
Control[a]	109.9 ± 13.0[b]	83.3 ± 13.6	77.1 ± 13.8
0.03	114.3 ± 12.7	95.4 ± 16.6	105.1 ± 12.4
0.1	116.7 ± 9.8	101.2 ± 19.9	119.6 ± 8.2
0.3	115.9 ± 13.9	110.4 ± 22.9	87.1 ± 6.4
1.0	104.5 ± 9.4	73.3 ± 14.6	26.4 ± 2.3[c]

[a] Cells treated with the carrier solvent in the medium.
[b] ng ATP/ml; average of 4 replicate samples ± SD.
[c] Significantly lower than the control, one-tailed t test, $P < 0.05$.

ED_{50} dose for phagocytosis, protein synthesis, and RNA synthesis was 0.09, 0.18, and 0.60 mM, respectively, after 2 hr exposure.

DISCUSSION

Penicillic acid is acutely toxic to rat alveolar macrophages *in vitro,* causing cell swelling, ^{51}Cr release, decreased ATP levels, inhibition of protein and RNA synthesis, and inhibition of phagocytosis in alveolar macrophage monolayer cultures.

In contrast to delayed mean cell volume changes induced by penicillic acid, there was significant ^{51}Cr release within 30 min exposure at a concentration of 0.1 mM penicillic acid. On the other hand, consistently significant mean cell volume increase required 2 hr exposure at 0.3 mM penicillic acid. The relative sensitivity of mean cell volume and ^{51}Cr release as indicators of cytotoxicity observed in these studies was consistent with our work with patulin reported earlier (Sorenson *et al.*, 1985). Although mean cell volume and ^{51}Cr release both reflect membrane transport properties, ^{51}Cr release is a direct effect of membrane

TABLE 4
INHIBITION OF PROTEIN SYNTHESIS IN ALVEOLAR MACROPHAGE CULTURES BY PENICILLIC ACID[a]

Concentration (mM)	1 hr		2 hr		4 hr	
	cpm ± SD[b]	% Control	cpm ± SD	% Control	cpm ± SD	% Control
Control[c]	434.4 ± 140.4	—	1143.1 ± 215.5	—	2723.3 ± 303.9	—
0.01	514.4 ± 49.9	118.4	1074.4 ± 181.8	94.0	2197.4 ± 116.1[d]	80.7
0.03	487.8 ± 62.6	112.3	971.5 ± 173.9	85.0	2125.8 ± 86.4[d]	78.7
0.1	454.9 ± 126.3	104.7	811.1 ± 181.6	71.0	1180.4 ± 229.1[d]	43.3
0.3	387.8 ± 102.9	89.3	440.9 ± 26.2[d]	38.6	281.1 ± 60.9[d]	10.3
1.0	239.1 ± 92.2[d]	55.0	147.2 ± 33.8[d]	12.9	99.6 ± 32.9[d]	3.7

[a] [^{3}H]Leucine incorporation.
[b] Counts per minute ± SD.
[c] Cells treated with the solvent carrier in the medium.
[d] Significant, $P < 0.05$.

TABLE 5
INHIBITION OF RNA SYNTHESIS IN ALVEOLAR MACROPHAGE CULTURES BY PENICILLIC ACID[a]

Concentration (mM)	1 hr		2 hr		4 hr	
	cpm ± SD[b]	% Control	cpm ± SD	% Control	cpm ± SD	% Control
Control[c]	7024.6 ± 2143.2	—	18,830.9 ± 1569.0	—	29,074.8 ± 3082.0	—
0.1	7404.7 ± 2010.2	105.4	16,858.7 ± 1300.3	89.5	26,172.1 ± 1730.0	90.0
0.3	7716.5 ± 1769.8	109.8	14,982.9 ± 1399.0[d]	79.6	12,918.8 ± 2370.0[d]	44.4
1.0	6290.4 ± 3190.1	89.5	5,122.6 ± 765.4[d]	27.2	4483.1 ± 743.1[d]	15.4

[a] [^{3}H]Uridine incorporation.
[b] Counts per minute ± SD.
[c] Cells treated with carrier solvent in the medium.
[d] Significant, $P < 0.05$.

damage, whereas change in mean cell volume occurs indirectly as a result of (1) leakage, (2) inhibition of the Na^+, K^+ pump (Na^+,K^+-ATPase), (3) decreased ATP, or (4) a combination of all of the above. 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 active outflux of Na^+ by the pump (Tosteson and Hoffman, 1960). Chan *et al.* (1979) reported that PA was a selective inhibitor of Na^+,K^+-ATPase in mouse brain and kidney tissue *in vitro*. ATP levels were significantly lowered by penicillic acid only after 4 hr exposure at 1.0 mM. In contrast, patulin produced a dose-dependent decrease in ATP levels after 1 hr exposure with significant inhibition ($P < 0.05$) at a concentration of 0.05 mM (Sorenson *et al.*, 1985) in the same *in vitro* system. Although rubratoxin B (Desaiah *et al.*, 1976) and patulin (Phillips and Hayes, 1977) inhibit oligomycin-sensitive Mg^{2+}-ATPase, penicillic acid did not (Chan *et al.*, 1979). The oligomycin-sensitive Mg^{2+}-ATPase complex has been linked to oxidative phosphorylation (Racker, 1975). Thus, the toxicity of PA may not relate directly to energy production.

Penicillic acid inhibited the synthesis of both protein and RNA synthesis al-

TABLE 6
EFFECT OF PENICILLIC ACID ON PHAGOCYTOSIS OF ^{51}Cr-LABELED SHEEP ERYTHROCYTES[a]

Concentration (mM)	cpm[b]	Percentage control
Control[c]	4230.0 ± 365.3	100
0.01	4152.8 ± 337.6	98.2
0.03	3340.4 ± 283.0[d]	79.0
0.1	2141.4 ± 209.2[d]	50.6
0.3	525.6 ± 93.2[d]	12.4
1.0	202.6 ± 45.0[d]	4.8

[a] Exposure time: 2 hr.
[b] Counts per minute; means of 5 replicate cultures ± SD.
[c] Cells treated with carrier solvent in the medium.
[d] Significantly different from controls, $P < 0.05$.

though protein synthesis was affected to a greater degree. For example, there was significant inhibition of RNA synthesis after 2 and 4 hr exposure to 0.3 mM PA, whereas there was significant inhibition of protein synthesis at 0.3 and 0.01 mM after 2 and 4 hr exposure, respectively. Since the energy requirements for both classes of macromolecular synthesis are similar, the difference cannot be explained as a result of decreased ATP supply. Furthermore, protein and RNA synthesis were inhibited earlier than, and at concentrations of PA below the level required for detectable reduction of ATP. The difference in sensitivity of protein and RNA synthesis suggests that inhibition of RNA synthesis might result, at least in part, from depletion of essential synthetic enzymes, although direct inhibition of RNA synthesis cannot be excluded. Kawasaki *et al.* (1972) studied the effect of PA on macromolecular synthesis in HeLa cells after 1 hr exposure and reported that inhibition of DNA synthesis was "a little more prominent" than RNA and protein synthesis at 100 μg/ml (0.59 mM), although "a significant difference among the inhibition of their synthesis was scarcely proved." The data presented in Fig. 6 of their paper suggest that protein synthesis was more sensitive under those conditions (1 hr exposure at 0.59 mM PA) than RNA synthesis.

Phagocytosis was somewhat more sensitive to PA than protein synthesis. Conceivably, there could be a direct effect of PA on the membrane, unrelated to leakage, which affects the ability of the macrophage to phagocytize, or an effect of PA on microtubule function. It may also be possible that the observed inhibition of phagocytosis is the combined effect of PA on two or more metabolic and/or cellular functions.

When PA was injected into mice (ip), cysteine had a protective effect if given (ip) prior to, but not after PA administration (Chan *et al.*, 1980). The large difference in results between pre- and post-treatment with cysteine led these investigators to suggest the formation of short-lived active metabolite(s) which rapidly bind to target molecules, since cysteine administered after PA administration did not reverse the toxicity.

There are a number of similarities between PA and patulin. Both are carcinogenic polyketide lactone mycotoxins of similar molecular size (MW = 170 and 154, respectively) produced by *Penicillium* species. In addition, both mycotoxins are sensitive to sulfhydryl groups. Patulin appears to be slightly more toxic than PA (kawasaki *et al.*, 1972).

These studies have demonstrated that penicillic acid is highly toxic to pulmonary alveolar macrophages *in vitro*. Thus inhalation of workplace aerosols contaminated with penicillic acid could have major deleterious effects on normal macrophage function.

REFERENCES

Busby, W. F., Jr., and Wogan, G. N. (1981). Patulin and penicillic acid. *In* "Mycotoxins and Other Fungal Related Food Problems" (J. V. Rodricks, Ed.), Chap. 6. Amer. Chem. Soc., Washington, D.C.

Castranova, V., Bowman, L., and Miles, P. R. (1979). Transmembrane potential and ionic content of rat alveolar macrophages. *J. Cell. Physiol.* **110**, 471–479.

Chan, P. K., Phillips, T. D., and Hayes, A. W. (1979). Effect of penicillic acid on adenosine triphosphatase activity in the mouse. *Toxicol. Appl. Pharmacol.* **49**, 365–372.

Chan, P. K., Reddy, C. S., and Hayes, A. W. (1980). Acute toxicity of penicillic acid and its interaction with pentobarbital and other compounds. *Toxicol. Appl. Pharmacol.* **52**, 1–9.

Ciegler, A., Detroy, R. W., and Lillehoj, E. B. (1971). Patulin, penicillic acid, and other carcinogenic lactones. *In* "Microbial Toxins" (A. Ciegler, S. Kadis, and S. J. Ajl, Eds.), Vol. 4, pp. 409–434. Academic Press, New York.

Desaiah, D., Hayes, A. W., and Ho, I. K. (1976). Effects of rubratoxin B on adenosine triphosphatase activities in the mouse. *Toxicol. Appl. Pharmacol.* **349**, 71–79.

Dickens, F., and Jones, H. E. H. (1961). Carcinogenic activity of a series of reactive lactones and related substances. *Brit. J. Cancer* **15**, 85–100.

Dickens, F., and Jones, H. E. H. (1965). Further studies on the carcinogenic action of certain lactones and related substances in the rat and mouse. *Brit. J. Cancer* **19**, 392–403.

Gerberick, G. F., and Sorenson, W. G. (1983). Toxicity of T-2 toxin, a *Fusarium mycotoxin*, to alveolar macrophages *in vitro*. *Environ. Res.* **32**, 269–285.

Gerberick, G. F., Sorenson, W. G., and Lewis, D. M. (1984). The effects of T-2 toxin on alveolar macrophage function *in vitro*. *Environ. Res.* **33**, 246–260.

Godin, D. V., and Schier, S. L. (1972). Modification of the erythrocyte membrane by sulfhydryl group reagents. *J. Membr. Biol.* **7**, 285–312.

Hayes, A. W. (1977). Effect of the mycotoxin, penicillic acid, on *Tetrahymena pyriformis*. *Toxicon* **15**, 497–504.

Kawasaki, I., Toshikazu, O., Umeda, M., and Saito, M. (1972). Cytotoxic effect of penicillic acid and patulin on HeLa cells. *Japan. J. Exp. Med.* **42**, 327–364.

Kurtzman, C. P., and Ciegler, A. (1970). Mycotoxin from a blue-eye mold of corn. *Appl. Microbiol.* **20**, 204–207.

Lipscomb, M. F., Toews, G. B., Lyons, C. R., and Uhr, J. W. (1981). Antigen presentation by guinea pig alveolar macrophages. *J. Immunol.* **126**, 286–291.

Murnaghan, M. T. (1946). The pharmacology of penicillic acid. *J. Pharmacol. Exp. Ther.* **88**, 119–132.

Myrvik, Q. N., Leake, E. S., and Faris, B. (1961). Studies on pulmonary alveolar macrophages from the normal rabbit: A technique to procure them in a high state of purity. *J. Immunol.* **86**, 126–132.

Natori, S., Sakaki, S., Kurata, H., Udagawa, S., Ichinoe, M., Saito, M., Umeda, M. (1970). Chemical and cytotoxicity survey on the production of ochratoxins and penicillic acid by *Aspergillus ochraceous* Wilhelm. *Chem. Pharm. Bull.* **18**, 2259–2268.

Phillips, H. J. (1973). Dye exclusion test for cell viability. *In* "Tissue Culture Methods and Applications" (P. R. Kruse and M. K. Patterson, Eds.), pp. 406–408. Academic Press, New York.

Phillips, T. D., and Hayes, A. W. (1977). Effects of patulin on adenosine triphosphatase activities in the mouse. *Toxicol. Appl. Pharmacol.* **42**, 175–187.

Phillips, T. D., and Hayes, A. W. (1979). Inhibition of electrogenic sodium transport across toad urinary bladder by the mycotoxin patulin. *Toxicology* **13**, 17–24.

Racker, E. (1975). Reconstitution, mechanism of action, and control on ion pumps. *Biochem. Soc. Trans.* **3**, 785–802.

Rega, A. F., Rothstein, A., and Weed, R. (1967). Erythrocyte membrane sulfhydryl groups and the active transport of cations. *J. Cell. Physiol.* **70**, 45–52.

Singh, J. (1967). Patulin. *In* "Antibiotics" (D. Gottlieb and P. D. Shaw, Eds.), Vol. 1, pp. 621–630. Springer-Verlag, Berlin/Heidelberg/New York.

SAS Institute. "Statistical Analysis System." Raleigh, N.C.

Snow, J. P., Lucas, G. B., Harvan, D., Pero, R. W., and Owens, R. G. (1972). Analysis of tobacco and smoke condensate for penicillic acid. *Appl. Microbiol.* **24**, 34–36.

Snydermann, R., Pike, M. C., Fischer, D. G., and Koren, H. S. (1977). Biologic and biochemical activities of continuous macrophage cell lines P388D1 and J774.1. *J. Immunol.* **119**, 2060–2066.

Sommerville, D., Van Scott, M. R., Miles, P. R., and Castranova, V. (1984). Heterogenicity of alveolar macrophages: Relationship between properties and lavage number. *Amer. Rev. Respir. Dis.* **129**, A6.

Sorenson, W. G., Simpson, J., and Castranova, V. (1985). Toxicity of the mycotoxin patulin for rat alveolar macrophages. *Environ. Res.* **38**, 407–416.

Sutherland, R. M., Rothstein, A., and Weed, R. J. (1967). Erythrocyte membrane sulfhydryl groups and cation permeability. *J. Cell Physiol.* **69**, 185–198.

Tosteson, D. C., and Hoffman, J. F. (1960). Regulation of cell volume by active cation transport in high and low potassium sheep red cells. *J. Gen. Physiol.* **44**, 169–194.

Ueno, Y., Matsumoto, H., Ishii, K., and Kukita, K. I. (1976). Inhibitory effects of mycotoxins on Na^+-dependent transport of glycine in rabbit reticulocytes. *Biochem. Pharmacol.* **25**, 2091–2095.

Umeda, M. (1971). Cytomorphological changes of cultured cells from rat liver, kidney, and lung induced by several mycotoxins. *Japan. J. Exp. Med.* **41**, 195–203.

Umeda, M., Tsutsui, T., and Saito, M. (1977). Mutagenicity and inducibility of DNA single-strand breaks and chromosome aberrations by various mycotoxins. *Gann* **68**, 619–625.

Umeda, M., Yamamoto, T., and Saito, M. (1972) DNA-strand breakage of HeLa cells induced by several mycotoxins. *Japan. J. Exp. Med.* **42**, 527–535.

Vincent, P. C., and Blackburn, C. R. B. (1958). The effects of heavy metal ions on the human erythrocyte. I. Comparison of the action of several heavy metals. *Aust. J. Exp. Biol.* **36**, 471–478.

Wilson, D. M. (1976). Patulin and penicillic acid. *In* "Mycotoxins and Other Fungal Related Food Problems" (J. V. Rodricks, Ed.), Advances in Chemistry Series No. 149, pp. 90–109. Amer. Chem. Soc., Washington, D.C.