

HEALTH IMPLICATIONS OF THE MOUNT ST. HELENS' ERUPTION: LABORATORY INVESTIGATIONS

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Abstract—A dry sedimented sample of Mount St. Helens volcanic ash was subjected to mineralogical and *in vitro* and *in vivo* biologic tests in order to define its potential for harm to exposed worker populations. The majority of the ash particles were within the respirable range and belonged to the plagioclase class of minerals. Free crystalline silica constituted 7.2% of the sample. *In vitro* biologic tests using varying concentrations of ash indicated the ash to be mildly fibrogenic. This was confirmed *in vivo*. Ash extracts were not mutagenic in two microbial assay systems and particulate ash had no effect on interferon production by monkey kidney cell monolayers or on the human complement system *in vitro*. Marked inhibition of the antibacterial substance, superoxide anion, from zymosan and ash exposed alveolar macrophages, suggests volcanic ash may impair antibacterial host defence mechanisms. Long-term inhalation and prospective autopsy studies of the exposed population are in progress.

INTRODUCTION

MOUNT ST. HELENS blasted approximately 4 km³ of rock and ash into the atmosphere during its first eruption on 18 May 1980. Since that date there have been four additional major eruptions resulting in ash exposure to a population of more than 1 million people (BATTELLE MEMORIAL INSTITUTE REPORT, 1980). In the most severely affected areas, the U. S. Environmental Protection Agency (EPA) reported that levels of total suspended particulates in the air ranged as high as 30–35 mg/m³ (CDC, 1980). A continuing problem is the redispersion of settled dust by dry windy weather and by the activities of man. As a result, certain occupational groups such as loggers, farmers, highway maintenance engineers and others may be exposed to greater concentrations of ash than the general population. The health implications of these exposures are difficult to assess, as there have been few precedents for this kind of disaster. Therefore, to deal promptly and effectively with environmental and occupational problems that might arise from these eruptions, the Center for Disease Control's (CDC) National Institute for Occupational Safety and Health (NIOSH) has initiated a multifaceted research project consisting of epidemiological and pathological studies of the affected population as well as *in vitro* and *in vivo* experimental studies. The following studies assessed the mutagenic, fibrogenic, and cytotoxic potential of the ash, together with its likely effect(s) on immunological, antiviral, and antibacterial defence mechanisms.

METHODS AND RESULTS

Ash used in the laboratory-based studies was a dry, sedimented sample from the first volcanic eruption, collected at Spokane (a city 340 km away from Mt. St. Helens that suffered a high dust fall-out). The ash was analysed by scanning electron microscopy combined with a LeMont B-10 automated image analysis system. Counts on 24 fields at a magnification of 2000 showed that 99% of the particles were within the respirable range, i.e. <10 μm in diameter. This respirable fraction constituted 81% by weight. Figure 1 shows the typical morphology of the ash sample. The majority of the particles had an angular profile; occasional porous pumice particles were also seen. Mineralogic analysis using X-ray powder diffraction, TEM combined with energy dispersive X-ray analysis (EDX), and light microscopy indicated a large number of particles belonged to the plagioclase mineral class (sodium, calcium and aluminium silicates). Other minerals identified included volcanic glass, albite, oligoclase, bytownite, labradorite, titanomagnetite, hornblende and quartz. Total crystalline silica and silica polymorphs were determined by X-ray powder diffraction on both the original sample and on a phosphoric acid digested residue of the ash. Total crystalline silica in the sample was 7.2%, of which 4.2% was cristobalite and 3.0% was quartz. The Talvite Colorimetric method was used to verify the total crystalline silica content (TALVITIE, 1951). No fibrous minerals were identified.

To assess the mutagenic potential of the ash, 50 g samples were extracted either with dichloromethane (DCM) or a mixture of ethyl acetate plus methanol for 16 h at 25°C with vigorous shaking. Extracts were collected by filtration and concentrated to dryness with a rotary evaporator. Each dried extract was dissolved in 4 ml dimethyl sulfoxide (DMSO) and tested with the Ames Salmonella *his* reversion assay (AMES *et al.*, 1975) and with the arabinose resistant forward mutation assay, using the plate incorporation test (PUEYO, 1978). The results are shown in Tables 1 and 2. There was no mutagenic activity over a wide range of concentrations (0.046–1.252 g/ash/plate) in either system. However, a moderate killing effect of the ash extract was noted at higher doses with the arabinose resistant assay.

Interferon is an important component of human antiviral defence mechanisms. Certain inorganic dusts are known to depress viral interferon induction *in vitro* (HAHON and ECKERT, 1976; HAHON *et al.*, 1980). Interferon induction by UV-irradiated PR8 influenza virus was determined in LLC-monkey kidney cell monolayers that had been pretreated with either 1, 2, or 4 mg of ash/ml. At the concentrations tested, ash was not cytotoxic to the cell monolayers (Table 3). Viral induced interferon production in cultures pretreated with ash was not adversely affected (Table 4). The ability of interferon to confer antiviral cellular protection was also not deterred by the presence of ash.

It has been suggested that the direct activation of complement with the formation of leukotactic factors is important in the pathogenesis of organic and/or mineral dust pneumoconiosis (OLENCHOCK *et al.*, 1980a, b; HASSELBACHER, 1979). Therefore, we investigated the effects of volcanic ash on the human complement system. Volcanic ash, in concentrations ranging from 0.2 to 40 mg/ml, was tested against human complement for Factor B activation, C₃ to C₃b conversion, and total hemolytic serum complement consumption (OLENCHOCK *et al.*, 1980a). Compared with the negative (silica flour) and positive (Durham wheat) controls, ash had a negligible effect on the alternative pathway. Over the range of concentrations used volcanic ash did not activate *in vitro*

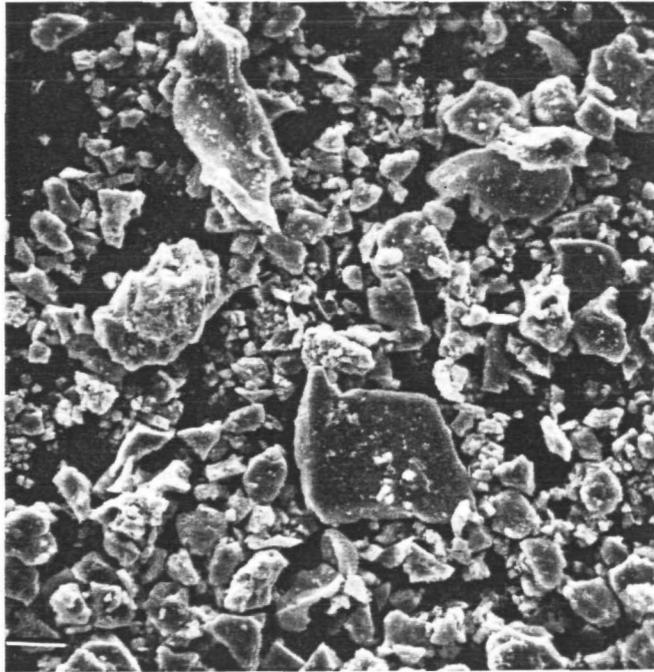


FIG. 1. SEM of Spokane volcanic ash sample. The majority of the particles were aluminum, calcium and sodium silicates. Marker = 10 μm . (ETEC Autoscan, 20 kV accelerating voltage.)

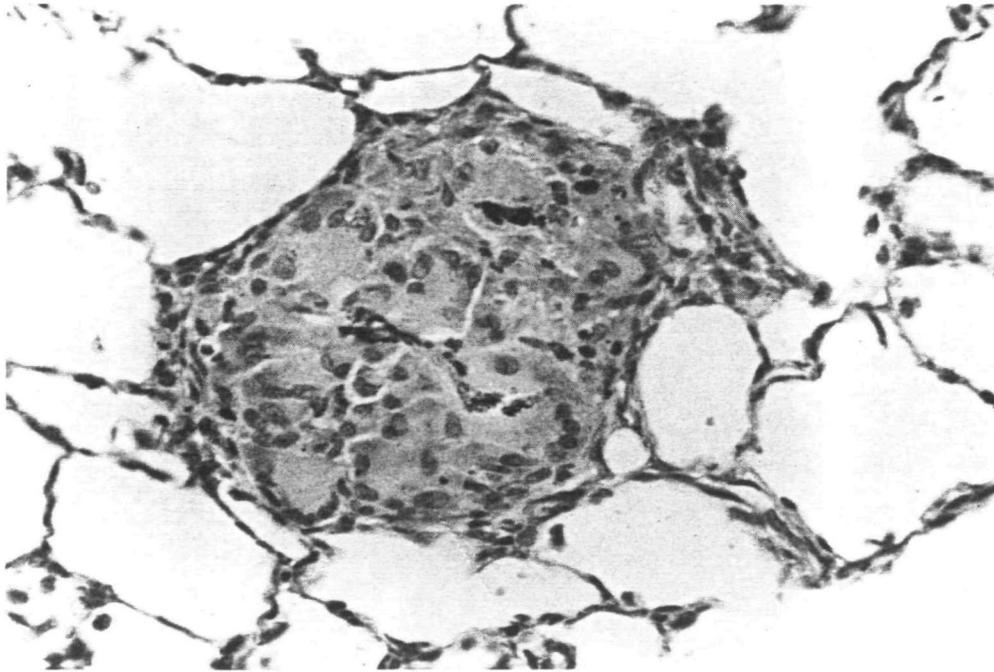


FIG. 5. Section of rat lung intratracheally injected with 10 mg volcanic ash and sacrificed at 28 days. An interstitial granulomatous lesion containing giant cells and ash particles is seen in centre of field. (Hematoxylin and Eosin $\times 400$.)

TABLE 1. REVERSION FREQUENCY OF *S. Typhimurium* TA 98 AND TA 100 TREATED WITH VOLCANIC ASH EXTRACTS IN THE PLATE INCORPORATION TEST*

Test compound	Volcanic ashes (g/plate)	Activation S-9	<i>his</i> + revertants/plate	
			TA 100	TA 98
Negative control (DMSO)		—	103	29
		+	130	36
Positive control (2-AA)†		—	127	32
		+	3107	2916
DCM extract	0.046	—	107	25
	0.139	—	108	29
	0.417	—	105	45
	1.252	—	99	36
	0.046	+	137	34
	0.139	+	128	40
	0.417	+	143	63
	1.252	+	140	74
E & M‡ extract	0.046	—	107	23
	0.139	—	111	25
	0.417	—	99	20
	1.252	—	99	20
	0.046	+	117	30
	0.139	+	110	26
	0.417	+	107	21
	1.252	+	109	35

* Aliquots (0.1 ml) of overnight bacterial culture and extract stock solution (prepared in DMSO) were plated onto an agar plate.

† '2-AA' means '2-aminoanthracene'.

‡ 'E & M' means 'ethyl acetate plus methanol'.

TABLE 2. MUTATION FREQUENCY OF *S. Typhimurium* SV-50 TREATED WITH VOLCANIC ASH EXTRACTS IN THE PLATE INCORPORATION TEST*

Test compound	Volcanic ashes (g/plate)	<i>ara</i> ' mutants/plate	
		Without activation	With activations
Negative control (DMSO)		141	179
Positive control (2-AA)†		165	1623
DCM extract	0.046	144	194
	0.139	134	205
	0.417	152	189
	1.252	76	146
E & M‡ extract	0.046	142	170
	0.139	140	197
	0.417	53	185
	1.252	18	157

* Aliquots of bacterial culture (10^7 cells) and extract stock solution (0.1 ml in DMSO) were plated.

† '2-AA' means '2-aminoanthracene'.

‡ 'E & M' means 'ethyl acetate plus methanol'.

TABLE 3. EFFECT OF DIFFERENT CONCENTRATIONS OF MT. ST. HELENS' ASH ON VIABILITY OF LLC-MK₂ (RHESUS MONKEY KIDNEY) CELLS

Ash concentration (mg/ml)	Surviving fraction of cells*		Mean
	Test number 1	Test number 2	
10	0.582	0.572	0.577
5	—	0.925	0.925
4	0.936	0.947	0.941
2	1.000	0.960	0.980
1	1.000	1.000	1.000
0 (control)	1.000	1.000	1.000

* Cell monolayers in 75 cm² flasks were treated with 10 ml of ash suspension for 24 h at 35°C. Results are expressed as surviving fraction of cells (trypan blue dye-exclusion) in ash-treated cell monolayers by the number of living cells test number 1 (8.6×10^6) and test number 2 (1.1×10^7) in control flasks.

TABLE 4. INTERFERON INDUCTION BY PR8 INFLUENZA VIRUS IN LLC-MK₂ CELL MONOLAYERS TREATED WITH DIFFERENT CONCENTRATIONS OF MT. ST. HELENS' ASH

Ash concentration* (mg/ml)	Interferon titer‡ (ICDD ₅₀ /ml)
4	100
2	98
1	100
0 (control)	105

* Ash suspended in 10 ml maintenance medium was added in different amounts to 75 cm² flasks containing approximately 10^7 cells per monolayer and incubated at 35°C for 24 h. Interferon was induced by adding 2 ml of UV-PR8 influenza virus onto cells which were then incubated at 35°C for 24 h. Assessment of interferon production was made by the immunofluorescent cell-counting technique. (HAHON *et al.*, 1975).

‡ Reciprocal of 50% infected cell-depressing dilution.

Factor B in pooled normal human serum. The ash did convert C₃ to C₃b as shown immunochemically by two-dimensional electrophoresis in gel. The degree of C₃ conversion was dose dependent, but only modest compared with that seen with Durham wheat. Hemolytic complement consumption ranged from 1.4 to 6.2% with ash compared with 3.8 to 7.5% for silica flour and 35.4 to 100% for Durham wheat. These studies show that volcanic ash has little effect on the human complement cascade *in vitro*.

Inhibition of mucoprotein synthesis in isolated rat trachea has been shown to be a useful and rapid assay for cytotoxicity (LAST *et al.*, 1977). Tracheal rings from pathogen-free Wistar Lewis rats were cultured in 4 ml modified McCoy's 5A medium. Four μ Ci D-(1-14C)-glucosamine hydrochloride (55.39 mCi/m mole) were added to

each culture followed by addition of concentrations of ash particulate ranging from 25 to 100 $\mu\text{g}/\text{ml}$. In addition, cultures were exposed to a DCM extract prepared from 5 g of the ash and to negative (DMSO) and positive (silica, coal tar extract) control substances. Media were removed at 24 hr and 10 ml of 10% cold trichloroacetic acid (TCA) was added. The precipitate was collected on 0.45 μm nitrocellulose filters. The filters were washed in cold TCA, then dissolved and the filtrates counted in a liquid scintillation counter. Results represent average of triplicate plates. ^{14}C -glucosamine incorporation was markedly reduced in ash extract exposed cultures (Table 5). However, no significant changes in ^{14}C -glucosamine incorporation were noted in particulate ash exposed cultures. These results indicate that ash extract, but not particulate ash, is moderately cytotoxic to rat tracheal mucus producing cells.

TABLE 5. EFFECT OF VOLCANIC ASH ON THE INCORPORATION OF ^{14}C -GLUCOSAMINE INTO ACID PRECIPITABLE MUCOPOLYSACCHARIDES

Compound	Dose	^{14}C counts/min*
DMSO	5 μl	586
Silica particles (80% 1–5 μm)	10 μg	270
Coal tar condensate†	5 μl	87
Volcanic ash (DCM extract)‡	10 μl	327
Volcanic ash (particulate)	100 $\mu\text{g}/\text{ml}$ 50 $\mu\text{g}/\text{ml}$ 25 $\mu\text{g}/\text{ml}$	620 575 598

* Triplicate plates. 4 tracheal rings per plate.

† Obtained by heating 20 g finely divided bituminous coal (to 450°) in a stream of dry N_2 . The condensate was collected on a Tenax GC column.

‡ Five g volcanic ash refluxed in DCM (10 ml) for 15 h, evaporated in N_2 and reconstituted with 50 μl DMSO. Each dish received 10 μl .

In vitro studies related to the fibrogenic properties of the ash were undertaken. The effect of volcanic ash on the growth of fetal lung fibroblasts (WI-38) was tested in concentrations of 1 to 500 μg ash/ml for 48 and 96 h (RYAN and CARDIN, 1967). At low concentrations, volcanic ash treated cultures showed a moderate increase in cell count (133% of control cultures) which was similar to that seen in silica exposed cultures (135% of control cultures) (Table 6). Monodisperse latex particles (1.1 μm in diameter) at the same concentrations had no effect on the growth of the fibroblasts.

It has been reported that rates of hemolysis produced by mineral dusts correlate with their fibrogenic potential (SUMMERTON and HOENIG, 1977). The ability of the volcanic ash to hemolyze sheep red cells was therefore studied over a range of concentrations (1.25–18.5 mg/ml) and compared with known fibrogenic and non-fibrogenic mineral dusts according to the method of HARRINGTON *et al.* (1971). At optimal concentration (12.5 mg/ml), the ash induced 60% hemolysis. Similar concentrations of talc (mildly fibrogenic), crystalline silica (very fibrogenic) and

TABLE 6. EFFECT OF VOLCANIC ASH ON GROWTH OF WI-38 FETAL LUNG FIBROBLASTS

Sample	Concentration	% of control culture*	
		48 h	96 h
Volcanic ash (99% < 10 μm diameter)	500 μg	94	103
	50 μg	91	116
	25 μg	96	128
	10 μg	98	126
	1 μg	98	133
Silica particles (80% < 1-5 μm diameter)	500 μg	98	111
	50 μg	103	122
	25 μg	100	133
	10 μg	96	126
	1 μg	101	135
Monodisperse latex particles (1.1 μm diameter)	200 μl	97	100
	100 μl	98	97
	50 μl	100	97
	20 μl	97	103
	10 μl	101	99

* Triplicate counts.

TABLE 7. HEMOLYTIC ACTIVITY OF VOLCANIC ASH IN COMPARISON WITH OTHER MINERAL DUSTS

Material	Hemolysis* (%)
Crystalline silica	96.1 \pm 0.8
Kaolin	84.4 \pm 4.2
Chrysotile	68.1 \pm 5.8
Mt. St. Helens volcanic ash	65.6 \pm 2.6
Talc	60.3 \pm 2.3
Coal dust	29.8 \pm 2.5
Bentonite	19.4 \pm 1.5
Amosite	13.1 \pm 1.6
Gypsum	4.8 \pm 1.4
Crocidolite	0.59 \pm 0.5

* Mean and 1 standard deviation for 6 replicate samples.

gypsum (non-fibrogenic) induced 60, 96 and 5% hemolysis respectively (Table 7).

Alveolar macrophages are phagocytic cells that play an important role in the defence of the lung against infectious agents and inhaled particles. When exposed to fibrogenic dusts, alveolar macrophages release lysosomal acid hydrolases, and this phenomenon may be used as an index of fibrogenic activity (SCHORLEMMER *et al.*, 1977). The selective release of lysosomal acid hydrolases was studied using rabbit alveolar macrophages. The macrophages were harvested from the lungs of male New Zealand white rabbits by lavaging with cold Ca^{++} and Mg^{++} free Hank's balanced salt solution using a modification of the method of MYRVIK *et al.* (1961). The cells were

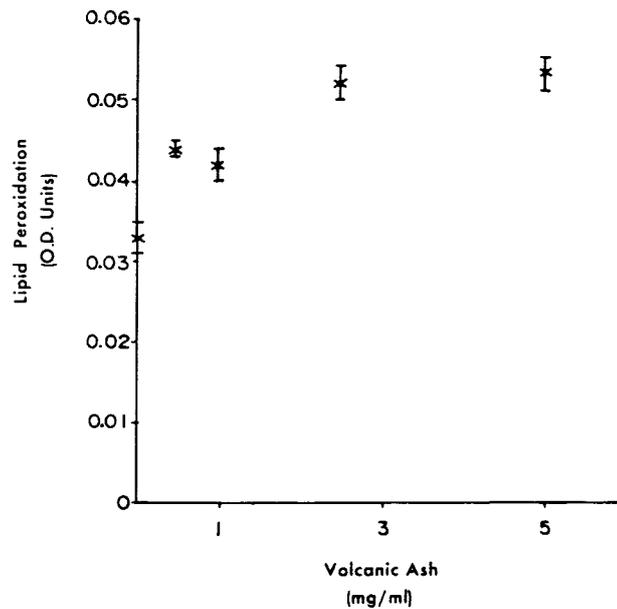


FIG. 2. The effect of *in vitro* exposure to various concentrations of volcanic ash on lipid peroxidation of rat lung microsomes. Lipid peroxidation was monitored by measuring malonaldehyde formation spectrophotometrically at 532 nm after a 2 h incubation at 37°C. The points represent mean value for three experiments and the bars indicate the SEM.

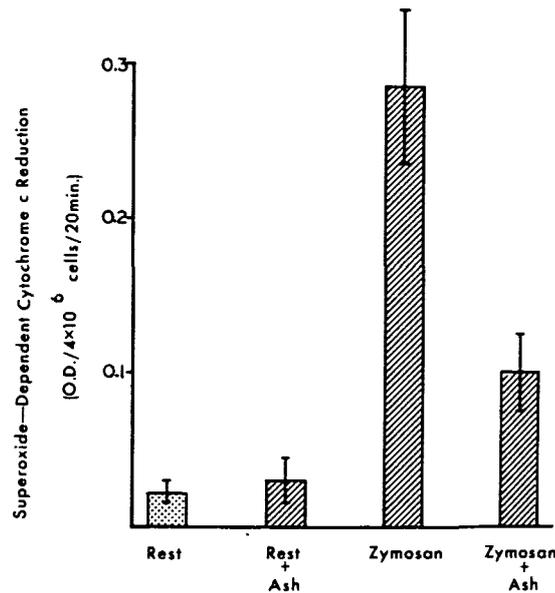


FIG. 3. The effect of *in vitro* treatment with 5 mg/ml volcanic ash on superoxide anion release from rat alveolar macrophages (4×10^6 cells) at rest and after exposure to zymosan particles (4 mg). Ash was added at 0 time to resting cells. Zymosan-stimulated cells were pre-incubated for 15 min with or without ash then zymosan was added at 0 time. Superoxide anion release at 37°C was measured spectrophotometrically at 550 nm for 20 min as the reduction of cytochrome *c*. Values are means \pm SEM of six experiments.

centrifuged, washed and viability determined by trypan blue dye exclusion. The cells were cultured in Medium 199 containing heat inactivated fetal calf serum, 100 units/ml penicillin, 100 $\mu\text{g/ml}$ streptomycin, 100 $\mu\text{g/ml}$ kanamycin and 10 $\mu\text{moles/ml}$ of L-glutamine. The cell suspension was prepared to contain 1.5×10^6 cells/ml of medium. Chrysotile asbestos (Duke standard) and volcanic ash were heat sterilized at 160°C for 90 min. The dusts were suspended in serum-free Medium 199 and sonicated for 2 min. Fetal calf serum was added to give a final concentration of 10% and the suspensions were incubated overnight at 37°C in 95% air and 5% CO_2 . Dust suspension was added to 15 ml cell suspension in 75 cm^2 Falcon flasks to give a final concentration of 50 $\mu\text{g/ml}$ of dust. The cultures were incubated for 24 h at 37°C in a 5% CO_2 atmosphere. At the end of the incubation period, the medium was decanted and the cells were removed using cold, Ca^{++} and Mg^{++} free, Hank's solution containing 0.1% Bovine Serum Albumin (w/v). The macrophages were again counted and the viability checked. After centrifugation the cell pellet was suspended in 4.0 ml of 0.25 M sucrose, freeze thawed, and sonicated. The homogenate was centrifuged at 25,000 r.p.m. for 30 min at 4°C . Protein content of the supernatant lysate was determined by the method of LOWRY *et al.* (1951). The following enzymes were assayed on the culture medium and lysate: lactate dehydrogenase, acid phosphatase (SOMMER, 1954), β -glucuronidase (FISHMAN *et al.*, 1967), β -galactosidase (CONCHIE *et al.*, 1959) and N-acetyl- β -D-glucosaminidase (KANG and SALVAGGIO, 1978). At a concentration of 50 $\mu\text{g/ml}$ of ash, no significant release of lysosomal enzymes was observed when compared with the positive control using chrysotile asbestos, a known inducer of fibrogenesis. Dose and time-dependent studies are currently in progress.

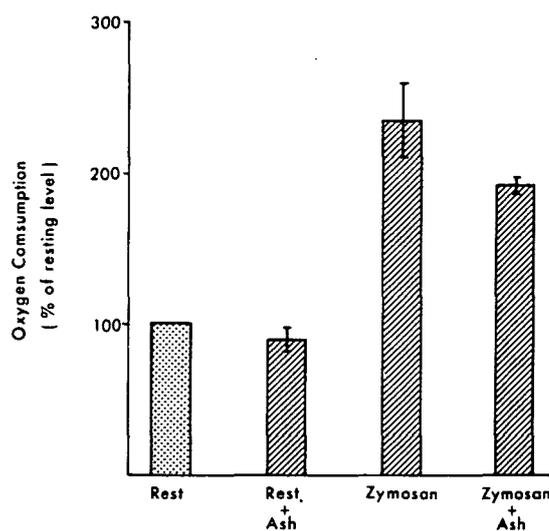


FIG. 4. The effect of *in vitro* treatment with 5 mg/ml volcanic ash on oxygen consumption in rat alveolar macrophages (4×10^6 cells) at rest after exposure to zymosan particles (4 mg). Ash was added at 0 time to resting cells. Zymosan-stimulated cells were pre-incubated for 15 min with or without ash, then zymosan was added at 0 time. Oxygen consumption was monitored for 10 min at 37°C using an oxygraph equipped with a Clark electrode. Oxygen consumption at rest is normally 7.7 ± 0.6 n moles/ 10^7 cells/min. Values are means \pm SEM of four experiments.

Lipid peroxidation is associated with pulmonary injury following exposure to toxic substances (CHOW and TAPPEL, 1973; BUS *et al.*, 1975). Therefore the effects of *in vitro* exposure to volcanic ash on the lipid peroxidation of rat lung microsomes was studied according to the method of WRIGHT *et al.* (1980). The results are shown in Fig. 2. The data indicate that exposure of lung microsomes to concentrations of volcanic ash ranging from 0.5 to 5 mg/ml did not result in significant peroxidation.

The effects of volcanic ash on a variety of cell physiology parameters have been studied according to previously published methods (CASTRANOVA *et al.*, 1979, 1980; MILES *et al.*, 1978). The membrane integrity of isolated rat alveolar macrophages was determined by their ability to exclude trypan blue dye, following incubation with 1.5 mg/ml^{-1} volcanic ash for 1 hr. In three experiments, no difference was noted in cell viability between ash-exposed and control cultures. The effects of ash exposure on alveolar macrophages was also determined by monitoring O_2 consumption and the release of the antibacterial substance—superoxide anion. Ash did not affect superoxide anion release (Fig. 3) or the amount of O_2 consumed (Fig. 4) by resting alveolar macrophages. However, ash markedly inhibited the increased superoxide anion release associated with zymosan exposure. Control experiments eliminated the possibility that this result was either an artifact due to ash reacting with the cytochrome C in the assay system or to the absorption of superoxide by the ash.

The toxicity of a respirable dust is often evaluated by its ability to induce fibrosis *in vivo*. In this regard, intratracheal injection provides a quick and simple technique for screening dusts. SPF Fisher 344 rats 10–12 weeks old, were intratracheally injected with 1 or 10 mg volcanic ash suspended in 0.25 ml sterile saline. Control animals received either an equal volume of saline or were air exposed. Animals receiving 10 mg volcanic ash IT were examined at intervals of 1(8), 7(4), 28(5), 56(2) and 180(10) days. Animals receiving 1 mg volcanic ash IT were only examined at 1(8), 56(8), and 180(8) days. The number of animals sacrificed is given in parentheses. Animals exposed to 10 mg of ash for 1 day showed a marked focal acute inflammatory cell response at the levels of the alveolar ducts and terminal bronchioles. This was also associated with deposition of ash at these sites. At 7 days, the reaction was still focal in nature; however, the inflammatory cell reaction was predominantly mononuclear in type. Animals examined at 28 and 56 days after exposure showed a mild interstitial fibrosis of the alveoli with occasional granulomas containing giant cells (Fig. 5). At 180 days, giant cell granulomata were observed in 9 of the 10 exposed animals. The lesions appeared to be larger than at the earlier periods and contained more reticulin and lymphocytes. None of the granulomas had hyalinized centres. Large quantities of ash were demonstrated in the lesions by polarizing microscopy and by backscattered electron imaging in the scanning electron microscope. The haphazard arrangement of the collagen fibres within the lesions more closely resembled the lesions associated with mixed mineral dust exposure than those due to pure silica. Animals exposed to 1 mg of ash showed similar but less pronounced reactions. Similar lesions were not seen in the lungs of control animals.

The pharmacological behaviour of isolated tracheal smooth muscles from rats which received 10 mg ash (1 day) was studied *in vitro* using conventional organ bath techniques. Compared with saline instilled controls, there was no effect of ash on contractions elicited with acetylcholine or potassium chloride or on relaxation induced by isoproterenol. The tissues from treated rats were, however, three-fold less sensitive

to the contractile effect of 5-hydroxytryptamine, indicative of a subtle toxicity to airway smooth muscle.

CONCLUSION

Overall, the tests and assays reported here indicate the volcanic ash is moderately biologically active and cannot be considered to be inert. The hemolytic activity of the ash, its stimulatory effect on fetal lung fibroblasts, and the mild fibrosis seen in exposed animals suggest a mild potential of the ash to induce fibrosis in the human population. In addition, the inhibition of the antibacterial substance—superoxide anion—from alveolar macrophages could have implications with regard to the ability of ash exposed workers to combat respiratory infections. As interpretative extrapolation from laboratory acquired data is notoriously difficult, prospective long term studies of the exposed human population are indicated. We are currently assessing the dust's (ash) effects on the lungs by (1) studying workers exposed to high concentrations of ash and (2) monitoring medical examiner and coroner cases from volcanic ash exposed areas and comparing these with age and sex matched cases from other parts of the country. These prospective studies on the exposed human population, together with further experimental animal studies, are expected to explicate any potential pathologic responses to the volcanic ash.

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