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Counteraction of Poly(4-Vinylpyridine-*N*-Oxide) on the Depression of Viral Interferon Induction by Coal Dust

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The depressive activity of coal dust on interferon induction by influenza was markedly subverted when either coal dust or LLC-MK_2 cell monolayers were pretreated with poly(4-vinylpyridine-*N*-oxide). The polymer alone neither induced interferon synthesis, inhibited viral induction of interferon, influenced viral multiplication, nor affected cellular-induced resistance by interferon. Adsorption of the polymer to coal dust not only occurred at a more rapid rate than to cell monolayers, but also less polymer was required to pretreat coal dust than cell monolayers to achieve comparable amelioration of interferon production. The polymer effectively negated the adverse activity of coal dust particles, irrespective of the latter's size (<2.0 to 74.0 μm). Virus multiplication in the presence of coal dust-treated cell monolayers attained a level that was twofold higher than that noted with either polymer-pretreated coal dust or polymer-pretreated cell monolayers. Interferon production was almost completely inhibited in the presence of coal dust; pretreating coal dust or cells with the polymer abrogated this inhibitory activity of coal dust. It is tentatively suggested that coal dust particles per se directly interact with cell membranes to subvert interferon induction and that the formation of an adsorbed polymer layer on these complexes prevents their interaction.

The interferon system is an important component of the nonimmunological defense mechanisms of the body, and it is generally believed that interferon plays a role in recovery from acute primary viral infections (3). The production of interferon by alveolar macrophages and the passive protection conferred by interferon to other macrophages against viral infections suggest that cell resistance mediated by interferon may be an important determinant of respiratory tract defenses (1). Studies on the influence of diverse mineral dusts on the nonimmune systems of the host have disclosed some provocative relationships. Recently, studies on the induction of interferon by influenza virus revealed that this adaptive celular response was depressed, partially or completely, in coal dust-treated human or simian cell monolayers (12) and in asbestos-treated simian cells (N. Hahon, and H. L. Eckert, Environ. Res., in press). As a consequence, a higher virus growth level was attained in coal dust-treated and asbestos-treated cells than in untreated cells. The demonstration that cobalt sulfate inhibited interferon activity in vitro, resulting in stimulation of viral plaque formation, may be relevant in explaining the increased mortality noted in encephalocarditis virus-infected mice exposed to cobalt sulfate (11). Interferon-containing preparations have been shown to enhance the phagocytosis of carbon particles by mouse peritoneal macrophages maintained in vitro (18, 19). Miller and Zarkower (27) presented evidence suggesting that silica or carbon dust inhalation produces functional alterations not only in macrophages but also in T and B lymphocytes. Carbon dust inhalation was also found to cause a significant enhancement of [^{3}H]thymidine uptake of sensitized mouse spleen cell cultures by purified protein derivative of human tuberculin. This may be indicative of a change in the delayed-type hypersensitivity response to *Mycobacterium tuberculosis* H37Ra (28). That interferon production may represent one of the factors responsible for the depression of cell-mediated immune reactions, i.e., delayed-type hypersensitivity, during virus infection has been reported (7).

In recent years, pneumoconiosis research has been fostered by demonstrations of the ability of poly(vinylpyridine oxides) and other nonionic polymers to prevent the fibrogenic effects of silica in animals and to inhibit its cytotoxic activity in cell cultures (16). Schlipköter and Brockhaus (33) first demonstrated the protection conferred to cells against silica dust by

polyvinylpyridine and by less toxic oxides of the polymer (34). The latter were effective both in vitro and in vivo (35). Although numerous other active polymers have been found (10, 17, 26, 29), the presence of hydrogen-accepting groups (either *N*-oxides or pyridinum) is frequently associated with protection. That these polymers may be administered by either inhalation or parenteral injections and effectively inhibit the development of silicosis in experimental hosts (16) is a therapeutic advantage. The possible applicability of the poly(vinylpyridine oxides) and related polymers in chemoprophylaxis or therapy represents one of the most promising advances in the field of pneumoconiosis.

The protection afforded by polymers against the pathogenic activities of silica prompted an investigation on the interactions between poly(vinylpyridine oxides) and coal dust and their respective influence on interferon induction by influenza virus. Cell cultures were the experimental host system employed for this purpose because of their proven sensitivity and efficiency for assessing the activities of polymers against mineral dusts (16) and for their amenability to coal dust and viral induction of interferon (12). This report describes the conditions affecting the amelioration of viral interferon induction by poly(4-vinylpyridine-*N*-oxide) (PVPNO) in the presence of coal dust.

MATERIALS AND METHODS

Viruses. The Ao/PR8/8/34 influenza and parainfluenza 1 (Sendai) virus strains employed in this study were obtained from the American Type Culture Collection, Rockville, Md. Stock virus pools of each strain were prepared from chicken embryonated eggs in the manner described previously (13). Influenza and Sendai virus pools contained 1.0×10^8 and 5.0×10^9 cell-infecting units of virus per ml, respectively, when assayed by the immunofluorescent cell-counting procedure (13).

Cell cultures. Rhesus monkey kidney ($LLC\text{-}MK_2$) and human chang conjunctiva (clone 1-5c-4) cell lines obtained from the American Type Culture Collection were used for induction and assay of interferon, respectively. Cell lines were propagated in plastic tissue culture flasks (75 cm^2) with Eagle minimum essential medium containing 10% fetal calf serum and maintained with minimum essential medium plus 0.5% fetal calf serum.

Reagents. Bituminous coal dust from the Pittsburgh seam, Cambria County, Pa., ranging in size from 2 to 3.2 μm, was generally employed in experimental studies. In tests wherein coal particles of varied sizes were employed, classification of particles was accomplished with a BAHCO microparticle classifier (H. W. Dietert Co., Detroit, Mich.).

PVPNO and poly(2-vinylpyridine-*N*-oxide) were obtained from Polysciences, Inc., Washington, Pa. Coal dust samples and suspensions of the polymers in phosphate-buffered saline (PBS), pH 7.1, were sterilized in an autoclave at a pressure of 20 lb/in^2 (126 C) for 15 min.

Interferon induction. The procedure generally employed to study the effects of PVPNO and coal dust on interferon induction was carried out as follows: 10 mg of PVPNO suspended in a 10-ml volume of PBS was added to flasks (75 cm^2) containing complete $LLC\text{-}MK_2$ cell monolayers that were incubated at 35 C for 8 h. This was decanted and 50 mg of coal dust suspended in a 10-ml volume of maintenance medium was added to flasks. After incubation at 35 C from 2 to 4 h, the coal dust suspension was removed. A 2-ml amount of influenza virus, which had been inactivated by ultraviolet (UV) irradiation for 45 s at a distance of 3 inches (ca. 7.62 cm) and a wavelength of 253.7 nm, was added onto cell monolayers that were then incubated at 35 C for 2 h. The multiplicity of infection was approximately 10. Inoculum was removed and 10 ml of maintenance medium was added to each flask. After incubation at 35 C from 22 to 24 h, supernatant fluid was decanted and centrifuged at $100{,}000 \times g$ for 1 h and dialyzed against HCl-KCl buffer, pH 2.0, at 4 C for 24 h. Dialysis was continued against two changes of PBS, pH 7.1, at 4 C for 24 h. Fluids were passed through Millex filters (0.45 μm) (Millipore Corp., Boston, Mass.) to obtain sterile preparations. Samples were stored at −70 C until assayed for interferon activity. Controls consisting of cell monolayers that were untreated or treated with either PVPNO or coal dust were handled exactly as described above. The test preparations possessed the biological and physical properties ascribed to viral interferons (24).

Interferon assay. Samples of interferon were assayed in duplicate. An immunofluorescent cell-counting assay of interferon was employed that has been described previously (14). Interferon-treated cell monolayers were challenged with 1×10^4 cell-infecting units of Sendai virus, and infected cells were visualized by the direct fluorescent-antibody staining. The reciprocal of the interferon dilution that reduced the number of infected cells to 50% of the control served as the measure of interferon activity, i.e., 50% infected cell-depressing dilution ($ICDD_{50}$). With this assay system, 0.8 international reference human (69/19) interferon units assayed as 1 U (12).

RESULTS

Preliminary considerations. An initial experiment was performed to ascertain whether viral induction of interferon in the presence of coal dust could be ameliorated by first treating cell monolayers with nonionic polymers. In the presence of coal dust, interferon yields from cell cultures pretreated with PVPNO or poly(2-vinylpyridine-*N*-oxide) were approximately 53 and 31%, respectively, in contrast to an interferon yield of 14% from untreated cells. Although the polymers were antagonistic to the depression of viral interferon induction by coal dust, they neither affected the efficacy of the viral inducer nor stimulated interferon per se.

Because PVPNO appeared to be more efficient, it was employed in succeeding experiments.

The ability of cells pretreated with PVPNO to maintain their resistance to the adverse effects of coal dust on viral interferon induction was investigated. Cell monolayers were exposed to 10 mg of PVPNO for 24 h; the polymer was then decanted, and the cells were held, additionally, for 48 h in maintenance medium. Subsequently, the cell cultures were exposed to 50 mg of coal dust for 4 h and then to the viral inducer. Controls consisted of cells exposed to PVPNO alone, PVPNO and virus, coal dust and virus, and virus alone. Results indicated that PVPNO-treated cells retained their capability, 48 h later, to counteract the depressing effect of coal dust on interferon production. Interferon was produced in these cell cultures in amounts comparable to that of cell culture controls that has been exposed only to the viral inducer.

The ability of PVPNO to adhere to cell monolayers and coal dust was determined by the resistance of the complexes to washing with PBS. The adherency of PVPNO was measured by the effect of the resultant combinations on viral induction of interferon. Results (Table 1) show that the polymer adhered to cell monolayers more strongly than to coal dust. After five washes of the PVPNO-cell monolayer complex, the interferon yield was still greater than that of the coal dust control, whereas, after one wash of the PVPNO-coal dust complex, the interferon yield was equal to the coal dust control.

A standard interferon preparation was assayed on clone 1-5c-4 cell monolayers that had been pretreated with coal dust or PVPNO to determine whether interferon could induce cellular resistance against challenge virus, after these experimental treatments. Results revealed that pretreatment of cell monolayers with coal dust, PVPNO, or combinations of both did not influence the ability of interferon to confer cellular resistance against challenge virus.

PVPNO concentration and coal particle size. The concentration of PVPNO per 1×10^7 cells that effectively abrogates coal dust depression of interferon production was determined. Cell monolayers were first treated with PVPNO concentrations that ranged from 0.1 to 50 mg and then with 50 mg of coal dust. Subsequently, the viral inducer was added. Maximal amelioration of interferon production in the presence of coal dust was obtained with 10 mg of PVPNO under the designated experimental conditions (Table 2). A higher concentration of the polymer (20 mg) did not significantly enhance interferon yields. All concentrations of PVPNO tested did not affect the efficacy of the viral inducer.

The relationship between PVPNO and coal particle size on the viral induction of interferon

TABLE 1. *Ability of PVPNO to adhere to LLC/MK$_2$ cell monolayers and coal dust, as determined by the effect of resultant combinations on interferon induction by influenza virus*

Washings of complexes[a]	Interferon yield (%)	
	PVPNO-coal dust	PVPNO-cell monolayers
0	83.5	96.2
1	29.4	92.5
3	29.4	81.4
5	29.4	29.4
Coal dust control	29.4	14.8
Viral inducer control[b]	100.0	100.0

[a] Interaction of PVPNO-coal dust was carried out by treating 50 mg of coal dust with 10 mg of PVPNO at 25 C for 2 h. After centrifugation, coal dust pellets were washed with PBS for the designated times, suspended in 10 ml of maintenance medium, and added to untreated cell monolayers that were incubated at 35 C for 20 h. PVPNO-cell monolayer interactions were carried out at 35 C for 16 h. Cells were washed as designated, and 50 mg of untreated coal dust suspended in 10 ml of maintenance medium was added to the PVPNO-treated cell monolayers that were incubated at 35 C for 20 h. Viral induction of interferon was then carried out (see text).

[b] Viral inducer controls, representative of 100% interferon yield, were 850 for PVPNO-coal dust and 270 for PVPNO-cells, measured by $ICDD_{50}$ of interferon.

TABLE 2. *Effect of pretreatment of LLC-MK$_2$ cell monolayers with varied PVPNO concentrations on viral interferon induction in the presence of coal dust*[a]

PVPNO[b] (mg)	Coal dust (50 mg)	Interferon ($ICDD_{50}$)[b]	Interferon yield (%)
20	+	360	90.0
10	+	350	87.9
5	+	160	40.0
1	+	100	25.0
0.1	+	90	25.0
–	+	0[c]	0.0
20	–	400	100.0
10	–	390	97.5
5	–	400	100.0
1	–	390	97.5
0.1	–	400	100.0
–	–	400	100.0

[a] Incubation at 35 C with PVPNO for 8 h, coal dust for 4 h, UV-irradiated PR8 influenza virus for 2 h, and maintenance medium for 24 h.

[b] Reciprocal of $ICDD_{50}$ per 10 ml.

[c] Lowest dilution of interferon tested was 1:5.

was investigated by introducing onto untreated and PVPNO-treated cell monolayers coal particles that ranged in size from <2.0 to a maximum of 74.0 μm. Interferon production in untreated cell monolayers was markedly influenced by the size of coal particles (Fig. 1). Coal particles 19.1 μm or smaller depressed interferon yields as much as fourfold when compared with that of controls; coal particles greater than 19.1 μm did not markedly affect interferon production. Pretreatment of cell monolayers with PVPNO effectively negated the depression of interferon induction by coal dust particles irrespective of the latter's size.

Time relationship between administration of PVPNO and coal dust. To determine whether the sequence of PVPNO and coal dust addition onto cell monolayers influenced viral interferon induction, cell cultures were treated with PVPNO before, simultaneously, or after the administration of coal dust. Results (Table 3) indicate that pretreatment of cells with PVPNO for 8 h proved highly effective in improving interferon yields in the presence of coal dust. Lesser time periods of PVPNO treatment resulted in a progressive decline in interferon yields. PVPNO given simultaneously with or within 2 and 4 h after coal dust was ineffective in counteracting the adverse action of coal dust on interferon production.

Virus multiplication in relation to PVPNO and coal dust. The growth of PR8 influenza virus concomitant with interferon production was studied in cover slip monolayers of LLC-MK_2 cells (3×10^5) that were treated in five different ways: 5 mg of PVPNO for 24 h before the addition of coal dust (PVPNO-coal dust), 2 mg of coal dust for 16 h followed by PVPNO (coal dust-PVPNO), PVPNO alone, coal dust alone, and untreated cell cultures. The multiplicity of infection was approximately 10. The

TABLE 3. *Time relationship between PVPNO and coal dust treatment of LLC-MK_2 cell monolayers on interferon induction by influenza virus*

Hourly relation at zero time[a]		Interferon ($ICDD_{50}$)[b]	Interferon yield (%)
PVPNO (10 mg)	Coal dust (50 mg)		
−16	+	230	82.1
−8	+	230	82.1
−4	+	180	64.2
−2	+	160	57.1
−1	+	70	25.0
0	+	55	19.6
+2	+	50	17.8
+4	+	50	17.8
−	+	50	17.8
−16	−	280	100.0
−	−	280	100.0

[a] PVPNO added to cell monolayers before (−), simultaneous (0), or after (+) coal dust cell cultures incubated with coal dust at 35 C for 16 h, with UV-irradiated PR8 virus at 35 C for 2 h, and then with 10 ml of maintenance medium for 22 h.

[b] Reciprocal of $ICDD_{50}$ per 10 ml.

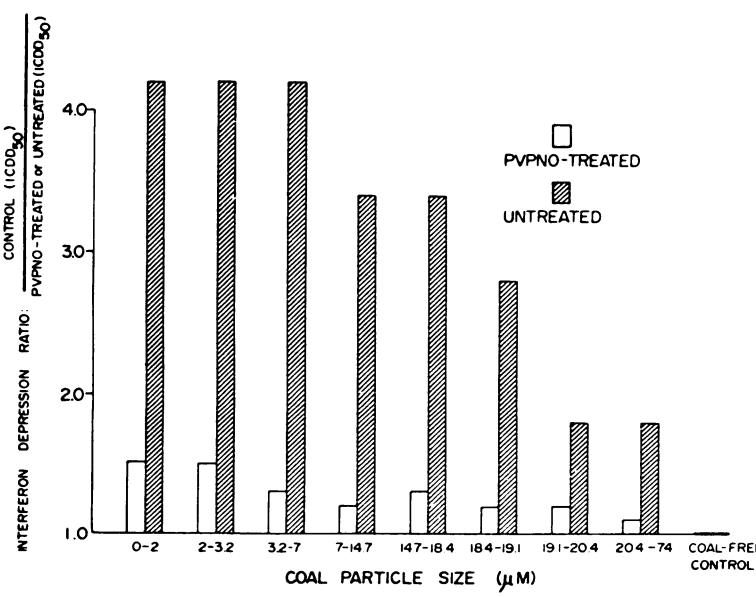

FIG. 1. *Relationship between pretreatment of LLC-MK_2 cell monolayers with PVPNO and coal dust particle size on depression of viral induction of interferon. Reciprocal of $ICDD_{50}$ of interferon for controls was 170.*

immunofluorescent cell-counting technique was used to determine virus multiplication from growth curve samples inoculated onto clone 1-5c-4 cells (13). For all experimental growth curve determinations, the rates of virus multiplication were similar (Fig. 2). However, in cell cultures treated with coal dust-PVPNO or coal dust alone, virus levels reached a plateau in 16 h that were twofold higher than that attained with PVPNO-coal dust-treated, PVPNO alone, or untreated cell monolayers. In samples of the latter three growth curves, interferon was first detected between 16 and 20 h. In cell cultures that were treated with coal dust-PVPNO or coal dust alone, only minimal amounts of interferon (less than 1 U/ml) were found in growth curve samples during the 48-h period. These data indicate that the slightly higher levels of virus growth attained in coal dust-treated cell cultures may be related to the depression of interferon synthesis. That cell cultures pretreated with PVPNO abrogated the action of coal dust, resulting in increased interferon production and decreased virus growth, further supports this view.

Direct interaction of coal dust with PVPNO on subsequent viral interferon induction. The experiments previously described were mainly concerned with the counteraction of PVPNO on viral interferon induction when the polymer was applied directly to cell monolayers before the addition of coal dust. The possibility that a similar effect may be evoked when PVPNO is first reacted with coal dust and then added onto cell monolayers prompted an investigation of this specified circumstance.

Mixtures of PVPNO and coal dust were incubated at 24 C for designated periods, and the absorption of the polymer by coal dust was determined by the effect of the resultant complex on viral interferon induction in cell monolayers. Results (Table 4) indicate that the interaction between PVPNO and coal dust was rapid and highly effective in preventing the adverse activity of coal dust on viral interferon production. Maximal adsorption of PVPNO by coal

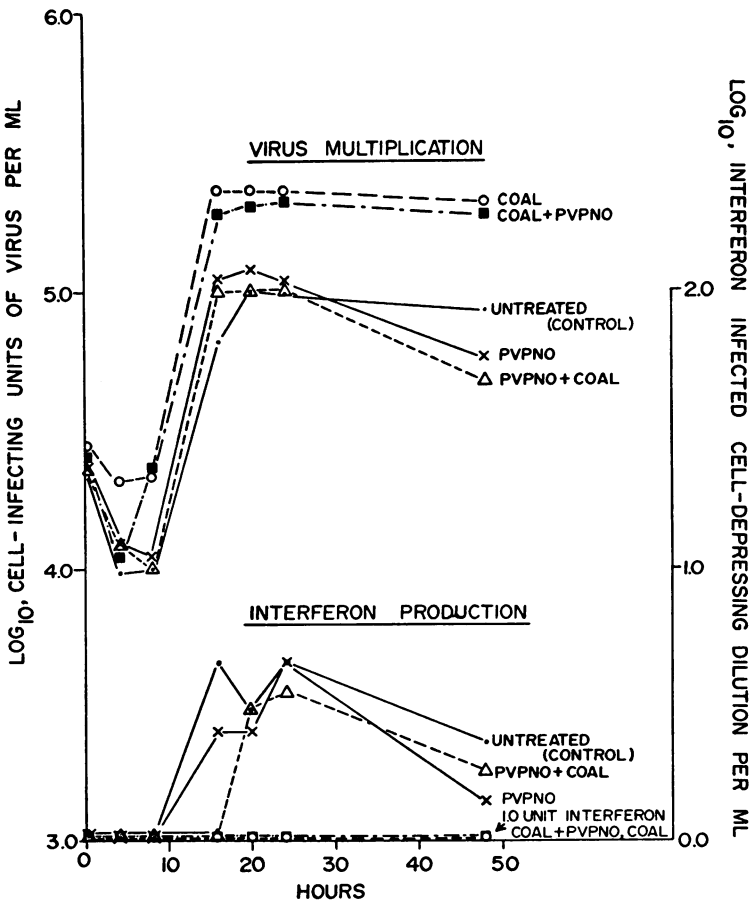

FIG. 2. *Growth curves of PR8 influenza virus concomitant with interferon production in LLC-MK$_2$ cell monolayers treated with PVPND before or after the addition of coal dust.*

TABLE 4. *Adsorption rate of PVPNO by coal dust, as determined by the effect of the resultant combination on interferon induction by influenza virus*

Incubation of PVPNO-coal dust mixtures[a] (h)	Interferon ($ICDD_{50}$)[b]	Interferon yield (%)
0.25	90	37.5
0.5	110	45.8
1.0	210	87.5
2.0	200	83.3
4.0	200	83.3
Controls		
Coal dust	50	20.8
Viral inducer	240	100.0
PVPNO	240	100.0

[a] Mixtures consisted of 10 mg of PVPNO and 50 mg of coal dust suspended in PBS, which were held at 24 C. At designated times, mixtures were centrifuged, and the coal dust pellet was resuspended in maintenance medium and added onto LLC-MK_2 cell monolayers. Coal dust and PVPNO controls were included. After incubation at 35 C for 16 h, suspensions were decanted, and cells were incubated at 35 C for 2 h with UV-irradiated PR8 virus and then with 10 ml of maintenance medium for 22 h.

[b] Reciprocal of $ICDD_{50}$ per 10 ml.

dust occurred within 1 h, as evidenced by the high interferon yield.

The quantity of PVPNO required to minimize the depression of interferon synthesis by coal dust when the reagents were directly interacted was determined. Quantities of PVPNO tested ranged from 0.01 to 20 mg. Results (Table 5) show that 10 mg of PVPNO completely abolished the depressive effect of 50 mg of coal dust on viral interferon induction. This procedure appeared to be slightly more effective in ameliorating interferon production than that of prior treatment of cell monolayers with the same quantity of PVPNO before the addition of coal dust (Table 2).

An experiment was carried out to ascertain the replication of influenza virus concomitant with interferon production in cell monolayers. This determination differed from the virus growth curve study described earlier in that coal dust was first pretreated with PVPNO at 35 C for 16 h before it was added onto cell monolayers. In all other aspects, the experiments were similar. For controls, virus growth was monitored in untreated and coal dust-treated cell monolayers. The results were comparable to those depicted in Fig. 2. In the present experiment, the rates of virus multiplication in untreated, PVPNO-coal dust-, and coal dust-treated cell monolayers were similar and reached a plateau in 16 h. However, virus growth in cell monolayers treated with coal dust attained a level that was twofold higher than that noted in untreated or PVPNO-coal dust-treated cell monolayers. Whereas interferon production was almost completely depressed in coal dust-treated cell monolayers, interferon was first detected at 16 h and, at subsequent time periods of 20, 24, and 48 h, in untreated and PVPNO-coal dust-treated cell monolayers. Pretreating coal dust with PVPNO effectively abolished the adverse activity of coal dust on interferon induction and, concomitant with interferon synthesis, virus growth was slightly diminished.

DISCUSSION

Under specified conditions, pretreatment of cell monolayers or coal dust with PVPNO diminished or abolished completely the depressive activity of coal dust on viral induction of interferon. These findings provide the basis for an additional indicator system to assess the protective activity of polymers against the adverse effects of mineral dusts on cell functions. PVPNO has been demonstrated to counteract the pathogenic effects of mineral dust (silica) for cell cultures by using the following indicators: cytotoxicity (4, 9), cell metabolism as measured by oxygen consumption (5, 25), rate of lactic acid synthesis (31), and leakage of acid phosphatase (21, 22). That the interferon system is responsive to the effects of mineral dust,

TABLE 5. *Prior treatment of LLC-MK_2 cell monolayers with complexes of coal dust and different quantities of PVPNO and their effect on viral induction of interferon*

Quantity of PVPNO incubated with coal dust[a] (mg)	Interferon ($ICDD_{50}$)[b]	Interferon yield (%)
20	280	100.0
10	280	100.0
5	250	89.2
2.5	220	78.5
1.0	180	64.2
0.5	180	64.2
0.25	130	46.4
0.1	120	42.8
0.05	60	21.4
0.01	50	17.8
0.00	50	17.8
Controls		
Viral inducer	280	100.0
PVPNO	280	100.0

[a] Mixtures consisted of 50 mg of coal dust and designated quantities of PVPNO suspended in PBS, which were incubated at 24 C for 2 h. Mixtures were centrifuged, and the coal dust pellet was resuspended in maintenance medium and added onto cell monolayers. After incubation at 35 C for 20 h, suspensions were decanted, and cells were incubated at 35 C for 2 h with UV-irradiated PR8 virus and then with 10 ml of maintenance medium for 22 h.

[b] Reciprocal of $ICDD_{50}$ per 10 ml.

amenable to quantitative assessment, and an integral component of nonimmunological cellular defense against infectious agents makes it valuable indicator for studies on experimental infective pneumoconiosis.

PVPNO minimally affected both the interferon system and host cells. The polymer neither induced interferon synthesis, inhibited viral induction of interferon, nor influenced cellular-induced resistance by interferon against challenge virus. Cell monolayers pretreated with PVPNO retained their ability 48 h later to resist the adverse effects of coal dust on interferon synthesis. Consistent with this finding was the ability of PVPNO to adhere more strongly to cell monolayers than to coal dust, as measured by resistance to washing. This is in contrast to findings that PVPNO is bound to silica particles but not to the red cell surface (29). The degree of polymer binding may vary with the test mineral-cell host system (36).

The depressive activity of coal dust on viral-induced interferon synthesis was markedly diminished when either coal dust or cell monolayers were pretreated with PVPNO. This is analogous to the findings of Kaw et al. (21) who reported that pretreatment of guinea pig macrophages with PVPNO before quartz exposure and coating of quartz with the polymer prevented almost completely the leakage of acid phosphatase enzyme. A difference was noted in the length of reaction time between the polymer and coal dust cr cells to attain comparable amelioration of interferon synthesis. The maximal effect was achieved by pretreating coal dust for 1 h with the polymer; however, cell monolayers had to be pretreated for 8 h. The procedure of pretreating coal dust with the polymer was not only more rapid than pretreating cell monolayers, but was twice as effective in terms of the quantity of PVPNO required to ameliorate interferon production in the presence of coal dust. For example, the interferon yield attained when 50 mg of coal dust was pretreated with 5 mg of PVPNO was comparable to that of cell monolayers pretreated with 10 mg of PVPNO and, subsequently, exposed to 50 mg of coal dust. Preliminary studies have revealed that the quantity of PVPNO required to protect the interferon induction process from the depressive activity of mineral dust may be dependent on the type of mineral; i.e., for protection against equivalent amounts of mineral dust, approximately 5,000 times more PVPNO was required for asbestos fibers than for coal dust (N. Hahon, unpublished data). Differences have been noted both in the quantity and the time factor adsorption of PVPNO by various mineral dusts (36).

The ability to depress viral induction of interferon was dependent on coal dust concentration and on particle size (12). Maximal and equivalent inhibition of interferon production was attained with coal dust particle sizes ranging from <2.0 to 19.1 μm. The size of these particles encompasses the range found in respirable coal mine dust (6, 29). Coal dust particles greater than 19.1 μm did not markedly affect interferon production. Pretreatment of cell monolayers with PVPNO effectively negated the depression of interferon induction by coal dust particles, irrespective of the latter's size.

Virus multiplication in the presence of coal dust-treated cell monolayers attained a level that was twofold higher than that noted with either untreated, PVPNO-pretreated coal dust, or PVPNO-pretreated cell monolayers. Whereas interferon production was almost completely depressed in coal dust-treated cell monolayers, pretreating coal dust or cells with PVPNO counteracted this adverse activity of coal dust. These data, in common with previous findings (12), infer that the higher level of virus growth attained in coal dust-treated cell cultures may be the consequence of diminished interferon production. It seems possible that a greater contrast in virus growth levels may be demonstrated between cells treated with only coal dust and cells protected with PVPNO if more potent interferon-inducing agents were used, i.e., Newcastle disease virus or group A arbovirus.

Findings contrary to the depressive role of mineral dust on interferon production have been reported (33). When silica was injected intravenously into mice to determine its effect on interferon induction by statolon and by Newcastle disease virus, the mineral had no inhibitory effect on interferon production by these inducers. It was suggested that subversion of interferon production does not play a significant role in the potentiation of virus infection in silica-treated animals. When various asbestos fibers or coal dust were treated with animal sera, the usual inhibitory activity of these mineral dusts on interferon production was abrogated (N. Hahon, unpublished data). Silica is known to adsorb serum proteins (20). The lack of a subversive effect by silica on interferon production may be explained by the mode of animal inoculation, i.e., injection of the mineral directly into the bloodstream, which resulted in the coating of silica with serum proteins.

In view of the complex composition of coal, defined as sedimentary rock mainly of vegetable origin, and its association with other minerals and siliceous material derived from rock

strata (37), it is difficult to either specify the elements or compounds that react directly with cell membranes to inhibit interferon synthesis or to delineate the mechanisms involved. Previous studies indicated that the adverse activity of coal dust on viral interferon induction was not related to either early virus-cell interactions, integration, virus replication, or the overt modification of cellular integrity (12). Furthermore, neither was interferon adsorbed to coal dust nor was a soluble ingredient or complex responsible. The evidence suggested that the phenomenon may be attributable to coal dust particles per se interacting with cell membranes. That various mineral dusts interact with cell membranes has been well documented (2). The cell surface interface is a complex, highly mobile structure that functions in recognizing certain extracellular materials and in transmitting information generated by their recognition to subcellular organelles (32). Changes in the metabolism of any one of the components of cell membranes (structural proteins, attached lipids, associated enzymes, or polysaccharide coat) induced by any one of a multiplicity of factors, i.e., hormones, drugs, or viruses, may mediate alterations in cellular function (8). Recently, it was demonstrated that alterations in cell surfaces of sensitive cells, which did not cause cell toxicity and were not metabolic in origin, inhibited the antiviral effect and induction response of the complex of polyinosinic and polycytidylic acids without modifying the binding function of the membrane receptors for the inducer (15, 30). There are certain similarities between these findings and those reported on the depression of viral interferon induction by coal dust (12). At present, our understanding as to how coal dust interposes in the inductive process of interferon synthesis remains to be elucidated.

The mechanism of action of poly(vinylpyridine-*N*-oxide) is also not well understood, although several theories have been proposed based, mainly, on experimental studies with silica. Theories regarding the mechanism of action of PVPNO have been reviewed by Holt (16). The protective action of the polymer has been attributed to (i) coating of the quartz surface, (ii) interaction with monosilicic acid formed by solution of silica in the cell, and (iii) stabilization of cellular or subcellular membranes. Part or all of these mechanisms may be involved in the protection afforded by PVPNO against silica but whether they are relevant to other systems may depend on several factors, i.e., types of mineral dust, cells, and pathological indicator response. Although other mechanisms are undoubtedly involved, the limited data available from this present study allow us only to suggest that the protective effect of PVPNO results from the formation of an adsorbed polymer layer on coal dust particles or on cell membrane receptors that prevents the interaction of these complexes and subverts the activity of coal dust on interferon induction.

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