Antagonistic activity of poly (4-vinylpyridine-N-oxide) to the inhibition of viral interferon induction by asbestos fibres

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ABSTRACT The depressive activity of both serpentine (Canadian and Rhodesian chrysotiles) and amphibole (amosite, crocidolite, and anthophyllite) asbestos fibres on interferon induction by influenza virus was significantly diminished or abolished completely when either asbestos fibres or LLC-MK₂ cell monolayers were pretreated with poly(4-vinylpyridine-N-oxide). Maximal antagonistic activity of the polymer was time and concentration dependent. Pretreating asbestos fibres with the polymer was more rapid and effective in encouraging viral interferon synthesis than pretreating cell monolayers. Virus multiplication in the presence of asbestos fibre-treated cell monolayers attained a twofold higher level than that noted in normal cell monolayers or those containing polymer-pretreated asbestos fibres. These findings were related to the suppression of interferon production.

The protection afforded by poly(vinylpyridine-N-oxides) and related polymers against the fibrogenic and cytotoxic activities of silica (Schlipkötter and Brockhaus, 1961; Beck *et al.*, 1963) is a promising advance in pneumoconiosis research that may have an application in the field of chemoprophylaxis (Holt, 1971). Although they are effective inhibitors of haemolysis by silica, the poly(vinylpyridine oxides) have little effect in preventing haemolysis by the serpentine form of asbestos (Macnab and Harington, 1967; Harington *et al.*, 1971). Under specified conditions, however, the polymer has been reported to counteract haemolysis by amphibole asbestiform fibres such as anthophyllite (Schnitzer *et al.*, 1971; Harington *et al.*, 1971). Although haemolysis of erythrocytes plays no part in the pathogenesis of pneumoconiosis, this *in vitro* phenomenon has been used to study the effects of mineral dusts on biological membranes (Harington *et al.*, 1975).

Mineral dusts may challenge the integrity and efficacy of the interferon system. Studies of the induction of interferon by influenza virus in cell

monolayers have shown that this adaptive cellular response was depressed, partially or completely, in the presence of either coal dust or serpentine and amphibole asbestos fibres (Hahon, 1974; Hahon and Eckert, 1976). Furthermore, virus multiplication was slightly enhanced in cell cultures pretreated with these mineral dusts. Because the interferon system is responsive to the effects of mineral dusts, amenable to quantitative assessment, and an integral component of nonimmunological cellular defence, it is a valuable indicator for studies on experimental infective pneumoconiosis at the cellular level.

A recent study has shown that the depressive activity of coal dust on viral interferon induction was markedly counteracted when either coal dust or cell monolayers were pretreated with poly (4-vinylpyridine-N-oxide) (PVPNO) (Hahon, 1976). The polymer alone neither induced interferon, inhibited viral induction of interferon, influenced virus replication, nor affected cellular-induced resistance by interferon. In view of the selective action of PVPNO in preventing haemolysis by certain asbestos fibres, the protection afforded by the polymer against the suppression, by both serpentine and amphibole asbestos fibres, of interferon synthesis in cell monolayers was investigated.

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Materials and methods

VIRUSES

The Ao/PR8/8/34 influenza and parainfluenza (Sendai) virus strains employed in this study were obtained from the American Type Culture Collection (ATCC), Rockville, Maryland. Stock pools of influenza and Sendai viruses, prepared from chick embryonated eggs, contained 5.0×10^8 and 5.0×10^9 cell-infecting units (CIU) of virus per millilitre, respectively, when assayed by the immunofluorescent cell-counting procedure (Hahon *et al.*, 1973).

CELL CULTURES

Rhesus monkey kidney (LLC-MK₂) and human Chang conjunctiva (clone 1-5c-4) cell lines obtained from ATCC were used for induction and assay of interferon respectively. Cell lines were propagated in plastic tissue culture flasks (75 cm²) with Eagle minimum essential medium (MEM) containing 10% fetal calf serum (FCS) and maintained with MEM plus 0.5% FCS.

REAGENTS

Union Internationale Contre le Cancer (UICC) asbestos fibre standards, amosite, anthophyllite, crocidolite, Canadian and Rhodesian chrysotiles were obtained from Duke Standards Co., Palo Alto, California. Optical microscope evaluations indicated that from 96 to 98% of the asbestos fibres were 30 μ m or less in length. Poly(4-vinylpyridine-N-oxide) was obtained from Polysciences, Inc., Warrington, Pennsylvania. The polymer and fibre samples, sterilised in an autoclave at 20 lb/in² (138.0 kPa) pressure (126°C) for 15 minutes, were made into w/v suspensions in either phosphate-buffered saline (PBS), pH 7.0, or maintenance medium.

INTERFERON INDUCTION

The procedure generally employed to study the interactions of PVPNO, asbestos fibres, and cells on interferon induction was carried out as follows: 100 mg of PVPNO suspended in 10 ml PBS was added to tissue culture flasks containing complete LLC-MK₂ cell monolayers which were incubated at 35°C for eight hours. After this pretreatment period, fluids were decanted and 1 mg asbestos fibres suspended in 10 ml maintenance medium was then added to the flasks. After incubation at 35°C for 16 hours, the asbestos fibre suspension was decanted. For pretreating asbestos fibres, a suspension consisting of 1 mg fibres and 100 mg PVPNO was held at 23°C for one to two hours. Asbestos fibres were then sedimented by centri-

fugation, resuspended in medium, and incubated with cell monolayers for the prescribed period.

For interferon induction, 2 ml of influenza virus that had been ultraviolet (UV)-irradiated for 45 seconds at a distance of 76.2 mm (3 in) and a wave length of 253.7 nm, were added to cell monolayers which were then incubated at 35°C for two hours. The multiplicity of infection (MOI) was approximately 10. Inoculum was removed and 10 ml maintenance medium was added to each flask. After incubation at 35°C from 22 to 24 hours, supernatant fluid was decanted, centrifuged at 100 000 *g* for one hour, and the fluid was then dialysed against HCl-KCl buffer, pH 2.0, at 4°C for 24 hours. Dialysis was continued against two changes of PBS, pH 7.1, at 4°C for 24 hours. Fluids were passed through 0.45 μ m Millex filters (Millipore Corporation, Bedford, Massachusetts) 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 asbestos fibres were handled exactly as described above. The test preparations possessed the biological and physical properties ascribed to viral interferons (Lockart, 1966).

INTERFERON ASSAY

Samples of interferon were assayed in duplicate. An immunofluorescent cell-counting assay of interferon was employed which has been described in detail elsewhere (Hahon *et al.*, 1975). Interferon-treated 1-5c-4 cell monolayers were challenged with 1×10^4 CIU of Sendai virus, and after the prescribed period of incubation, infected cells were visualised by direct fluorescent-antibody staining. The reciprocal of the interferon dilution that reduced the number of infected cells to 50% of the control, that is the ICDD₅₀ (50% infected cell-depressing dilution), served as the measure of interferon activity.

Results

THE EFFECTS OF TIME FACTORS, PVPNO AND ASBESTOS FIBRES ON INTERFERON PRODUCTION IN CELL MONOLAYERS

The optimal time of reaction between PVPNO and asbestos fibres or cell monolayers to prevent the suppression, by asbestos fibres, of viral interferon induction, was investigated. Five different asbestos fibres, amosite, anthophyllite, crocidolite, Rhodesian and Canadian chrysotiles, each mixed with PVPNO, were incubated at 23°C for periods ranging from 15 minutes to four hours. The extent of PVPNO interaction with asbestos fibres was measured by the effect of resultant combinations on viral interferon induction in cell monolayers.

Results (Table 1) indicate that PVPNO was rapidly adsorbed by the different asbestos fibres and that PVPNO was highly effective in preventing the adverse activity of the fibres on viral interferon production. The uniformly high and equivalent interferon yields indicated that maximal adsorption of PVPNO by all asbestos fibres tested occurred within 30 minutes. Interferon yields from cell cultures treated with PVPNO alone were equivalent to those from normal cell monolayers; interferon yields from cell monolayers treated with asbestos fibres were reduced by between 70 and 75%.

To determine whether the order of, and time interval between, the addition of PVPNO and asbestos fibres to cell monolayers was critical, cell cultures were treated with PVPNO before, at the same time as, or after, the addition of asbestos fibres. Results (Table 2) show that pretreatment of cell monolayers with PVPNO for the maximal time

period of 16 hours significantly limited the depressive effect of asbestos fibres on viral interferon production, although this period was insufficient to enable the polymer to nullify completely the depressive effect of asbestos fibres. Pretreatment of cells with PVPNO for shorter periods brought about correspondingly reduced interferon yields. PVPNO added at the same time as asbestos fibres, or two and four hours later, did not counteract their effect.

ADHERENCE OF PVPNO TO ASBESTOS FIBRES AND CELL MONOLAYERS

The adherence of the polymer to asbestos fibres, represented by Rhodesian chrysotile, or to cell monolayers was determined by the ability of the corresponding combinations to resist washing with PBS, as shown by the effect of the resultant combinations on viral interferon induction. Results (Table 3) indicate that the polymer adhered to

Table 1 Adsorption rates of PVPNO to asbestos fibres as determined by the effect of the resultant combination on interferon induction by influenza virus

Incubation of PVPNO-asbestos fibre-mixtures ¹ (h)	Asbestos fibres				
	Amosite	Anthophyllite	Crocidolite	Rhodesian chrysotile	Canadian chrysotile
0.25	60.9 ^a	73.1	65.8	68.2	63.4
0.5	100.0	100.0	95.1	100.0	97.5
1.0	100.0	95.1	100.0	100.0	100.0
2.0	97.5	97.5	100.0	97.5	97.5
4.0	100.0	100.0	95.1	100.0	97.5
Controls:					
Asbestos	26.8	26.8	29.2	29.2	26.8
PVPNO	100.0	100.0	97.5	100.0	97.5
Viral inducer ² (100.0)					

¹Mixtures consisting of 100 mg PVPNO and 1 mg asbestos fibres suspended in PBS were held at 23°C.

²UV-irradiated PR8 virus. Representative of 100% interferon yield was 410 ICDD₅₀.

^aPer cent interferon yield.

Table 2 Time factors in PVPNO and asbestos fibre treatment of LLC-MK₂ cell monolayers and subsequent interferon induction by influenza virus

Hourly relation between PVPNO and asbestos fibres ¹	Asbestos fibres				
	Amosite	Anthophyllite	Crocidolite	Rhodesian chrysotile	Canadian chrysotile
- 16	70.0 ^a	71.4	71.4	70.0	71.4
- 8	46.6	60.0	46.6	53.3	46.6
- 4	36.6	30.0	30.0	30.0	26.6
- 2	30.0	20.0	23.3	16.6	26.6
- 1	16.6	16.6	20.0	20.0	16.6
0	16.6	16.6	20.0	16.6	20.0
+ 2	16.6	16.6	20.0	16.6	16.6
+ 4	16.6	16.6	20.0	16.6	16.6
Controls:					
Asbestos fibres	16.6	16.6	20.0	16.6	16.6
PVPNO	100.0	100.0	100.0	100.0	100.0
Viral inducer (100.0) ²					

¹PVPNO added to cell monolayers before (-), at the same time as (0), or after (+) incubation of cell cultures with asbestos fibres.

²Representative of 100% interferon yield was 300 ICDD₅₀.

^aPer cent interferon yield.

Table 3 *Ability of PVPNO to adhere to asbestos fibres (Rhodesian chrysotile) and to LLC-MK₂ cell monolayers, determined by the effect of resultant combinations on interferon induction by influenza virus*

Washings of complexes ¹	Per cent interferon yield	
	PVPNO/chrysotile	PVPNO/cell monolayers
0	100.0	96.0
1	85.1	80.0
3	82.9	40.0
5	68.0	32.0
Controls:		
Chrysotile	18.0	32.0
Viral inducer ²	100.0	100.0

¹Cell monolayers and 1 mg chrysotile test samples were treated with 100 mg PVPNO at 35°C for 16 h and at 24°C for 2 h, respectively. Cell monolayers and sedimented chrysotile were washed with PBS for the designated times. PVPNO-treated chrysotile was suspended in 10 ml maintenance medium and added to untreated cell monolayers which were incubated at 35°C for 20 h. Untreated chrysotile was similarly added to PVPNO-treated cell monolayers. Viral induction of interferon was then carried out as described in Materials and methods.

²Representative of 100% interferon yield were 470 ICDD₅₀ for PVPNO/chrysotile and 250 ICDD₅₀ for PVPNO/cell monolayers.

asbestos fibres more strongly than to cell monolayers. After five washes, the PVPNO-asbestos fibre complex still encouraged a 50% greater interferon yield than did the corresponding asbestos fibre control, whereas when the PVPNO-cell monolayer complex had been washed three times the subsequent interferon yield was almost as low as that of the corresponding control.

The ability of PVPNO to adhere to cell monolayers for prolonged intervals and then to negate the adverse activity of various types of asbestos fibres on viral interferon induction was investigated. Cell monolayers were exposed to 100 mg PVPNO for 16 hours, the polymer was then decanted, and the cells were incubated for an additional 48 hours with maintenance medium. Subsequently, the cell

cultures were exposed to 1 mg of either amosite, anthophyllite, crocidolite, or chrysotile and then to the viral inducer. Controls consisted of cell monolayers exposed to PVPNO, PVPNO and virus, asbestos fibres and virus, and virus alone. Results indicated that PVPNO-treated cells retained their ability to counteract the depressing effect of each of the tested asbestos fibres on interferon production, for as long as 48 hours. Interferon was produced in these cell cultures in amounts equivalent to that of cell culture controls that had been exposed only to the viral inducer. The presence of PVPNO alone neither affected viral interferon production nor induced interferon *per se*.

PRETREATMENT OF ASBESTOS FIBRES AND CELL MONOLAYERS WITH VARIED PVPNO CONCENTRATIONS

In order to determine the concentration of PVPNO required to minimise the depression of interferon synthesis by pretreated asbestos fibres, from 1 to 100 mg PVPNO were incubated with 1 mg of five different asbestos fibres for one hour at 24°C. Results (Table 4) show that the maximal amount of 100 mg PVPNO was necessary to abolish almost completely the inhibitory activity of asbestos fibres on viral interferon production. PVPNO pretreatment of asbestos fibres was most effective with amosite and least with anthophyllite. Crocidolite, Rhodesian chrysotile, and Canadian chrysotile occupied intermediate positions. As little as 25 mg PVPNO suppressed the adverse effect of amosite on interferon production; however, PVPNO concentrations of 100 mg or less were insufficient to counteract completely the effect of anthophyllite.

When cell monolayers (1×10^7 cells) were pretreated with amounts of PVPNO similar to those employed in the previous experiment, 100 mg PVPNO were found to encourage interferon production to a significant extent in the presence of

Table 4 *Pretreatment of asbestos fibres with different quantities of PVPNO and its effect on viral interferon induction*

PVPNO ¹ (mg)	Asbestos fibres				
	Amosite	Anthophyllite	Crocidolite	Rhodesian chrysotile	Canadian chrysotile
100	100.0 ²	86.3	100.0	85.3	90.2
50	100.0	31.8	76.9	78.0	75.0
25	100.0	24.5	52.4	50.0	49.2
10	50.0	22.7	25.6	31.7	36.5
1	22.7	22.7	23.1	21.9	23.1
0	22.7	25.0	21.9	21.9	23.1
Control:					
Viral inducer ³	100.0				

¹Mixtures of asbestos fibres (1 mg) and designated quantities of PVPNO suspended in PBS were incubated at 24°C for 1 h and then centrifuged. Asbestos fibre pellets were resuspended in maintenance medium and added to cell monolayers which were incubated at 35°C for 16 h. Viral induction of interferon was then carried out as described in Materials and methods.

²Per cent interferon yield.

³Representative of 100% interferon yield was 820 ICDD₅₀.

Table 5 The effect of pretreatment of LLC-MK₂ cell monolayers with varied quantities of PVPNO on viral interferon induction in the presence of asbestos fibres

PVPNO ¹ (mg)	Asbestos fibres				
	Amosite	Anthophyllite	Crocidolite	Rhodesian chrysotile	Canadian chrysotile
100	76.6*	66.6	66.6	66.6	83.3
50	50.0	46.6	33.3	66.6	33.3
10	23.3	26.6	20.0	16.6	16.6
1	16.6	16.6	16.6	16.6	16.6
0	16.6	16.6	20.0	16.6	16.6
Controls:					
PVPNO (100 mg) without asbestos fibres 100.0					
Viral inducer ² (100.0)					

¹Cell monolayers incubated with PVPNO at 35°C for 8 h, 1 mg asbestos fibres for 16 h, UV-irradiated PR8 influenza virus for 2 h, and maintenance media for 24 h.
²Per cent interferon yield.
³Representative of 100% interferon yield was 300 ICDD₅₀.

the five different asbestos fibres, although the inhibitory effect of these fibres was not completely suppressed (Table 5). In contrast to the results obtained when the fibres themselves were pretreated, there was no clear distinction between the effects of PVPNO on the individual types of asbestos fibres.

VIRUS MULTIPLICATION

The growth of PR8 influenza virus and concomitant interferon production were studied in cover slip monolayers of LLC-MK₂ cells (3 × 10⁵). Cell monolayers were incubated for 16 hours with either Canadian chrysotile (0.25 mg) or chrysotile pretreated with PVPNO. Untreated cell cultures served as controls. Subsequently, virus inoculum was introduced on to all groups of cell monolayers at a multiplicity of infection of approximately 10. Growth curve samples were inoculated onto clone 1-5c-4 cells and virus replication was determined by the immunofluorescent cell-counting technique. Samples were assayed for interferon as previously described.

The rates of virus multiplication in untreated and both groups of treated cell monolayers were similar and reached a plateau in approximately 24 hours (see Figure). However, virus growth in cell monolayers treated with chrysotile alone attained a level that was twofold higher than that noted in untreated or PVPNO-chrysotile-treated cell monolayers. Whereas interferon production was almost completely suppressed in chrysotile-treated cell monolayers (less than one unit per ml), in untreated and PVPNO-chrysotile-treated cell monolayers interferon was detected as early as 16 hours after treatment was initiated and subsequently after 20, 24, and 48 hours. Pretreating chrysotile fibres with PVPNO effectively nullified their effect on interferon induction. These results suggest that the slightly higher level of virus growth attained in chrysotile-

Figure Growth curves of PR8 influenza virus and simultaneous interferon production in LLC-MK₂ cell monolayers previously treated with chrysotile or poly(4-vinylpyridine-N-oxide)-coated chrysotile fibres.

treated cell cultures may be related to suppression of interferon synthesis; pretreatment of chrysotile with PVPNO counteracted this suppression, and encouraged interferon production at the expense of virus growth.

Discussion

This study has shown that PVPNO abolishes or significantly diminishes the suppressive effects of both serpentine (Canadian and Rhodesian chrysotiles) and amphibole (amosite, crocidolite, and anthophyllite) asbestos fibres on viral induction of

interferon. The extent to which the polymer modifies the effects of asbestos fibres depends on factors such as whether asbestos fibres or cells are pretreated, PVPNO concentration, and length of treatment. Pretreatment of asbestos fibres has a greater and more rapid effect than pretreatment of cell monolayers. Within 30 minutes of pretreating asbestos fibres with the polymer, their effect on interferon production is nullified; however, 16 hours' pretreatment of cell monolayers has only a limited effect on the subsequent ability of asbestos fibres to suppress interferon induction in these cells, and shorter pretreatment has a minimal effect. The polymer is ineffective when added to cell monolayers after the addition of asbestos. These results are similar to those obtained with coal dust (Hahon, 1976), and analogous to those reported by Kaw *et al.* (1975) with quartz.

Prolonged incubation of cells with PVPNO increases their storage of the polymer (Allison, 1971). This may partly explain the prolonged incubation time required to pretreat cell monolayers with the polymer to achieve maximal protection. PVPNO, administered *in vivo* or *in vitro*, is stored in the lysosomes of cells (Grundmann, 1967; Bruch, 1967; Beck and Boje, 1967; Bairati and Castano, 1968); as it is resistant to hydrolytic enzymes, it remains there indefinitely (Allison, 1971). This is compatible with our finding, that PVPNO-treated cells are protected against the effect of asbestos fibres on interferon production for as long as 48 hours after removal of excess polymer.

Schnitzer (1974) demonstrated with haemolysis tests that PVPNO and other polymers reduce the lytic action of asbestos fibres and that adsorption of polymers by fibres is rapid and stable. In our experiments, the polymer adhered to asbestos fibres more strongly than to cell monolayers. In contrast, PVPNO adheres less strongly to coal dust than to cell monolayers (Hahon, 1976). PVPNO is readily bound to silica but not to the surface of red cells (Nash *et al.*, 1966). The degree, stability and rapidity of polymer binding varies with the nature of the mineral dust and type of cell being studied (Schnitzer, 1974; Hahon, 1976).

When asbestos fibres were pretreated with different amounts of PVPNO, a maximal concentration of 100 mg PVPNO per mg fibres was usually necessary to prevent inhibition of viral interferon production. However, the effect of amosite could be suppressed by as little as 25 mg PVPNO. Anthophyllite fibres were the most resistant to PVPNO. Suppression of the adverse effects of the five different types of asbestos fibres was only partially achieved when cell monolayers were pretreated with 100 mg PVPNO for eight hours. Under these conditions,

the effectiveness of the polymer did not differ markedly between different types of asbestos fibres. However, approximately 5000 times more PVPNO was required for protection against the effects of asbestos fibres than for protection against coal dust (Hahon, 1976). The quantity of PVPNO required to protect the interferon induction process from the depressive activity of mineral dust thus appears to be dependent on the nature of that dust.

Occupational respiratory carcinogens, arsenic and cobalt, have been reported to increase virus plaque formation and to inhibit the synthesis and/or action of interferon (Gainer, 1972a; Gainer, 1972b). In general concurrence with these findings and confirming the results of earlier studies concerned with virus replication in the presence of PVPNO-treated coal dust (Hahon, 1976), we found that influenza virus growth attained a level that was twofold higher in cell monolayers treated with chrysotile than in normal cell monolayers or those containing PVPNO-pretreated chrysotile. These results were related to the suppression of interferon synthesis in chrysotile-treated cell monolayers. PVPNO counteracted the inhibitory action of the asbestos fibres and thus allowed interferon production and virus multiplication to take place to an extent comparable to that in normal cell monolayers. The presence of PVPNO alone neither inhibited viral induction of interferon, induced interferon synthesis, nor influenced viral multiplication.

Theories regarding the mechanisms of action of PVPNO, based largely on studies with silica, have been reviewed by Holt (1971). Experimental studies with silica have shown that particles are taken up into cell lysosomes, and N-oxide groups can form strong hydrogen bonds with hydroxyl groups of silicic acid preventing the latter from attacking the lysosomal membranes and thereby averting the release of lysosomal enzymes (Comolli, 1967; Beck, 1970; Allison, 1971). There is evidence that cell membranes of lung fibroblasts in monolayer culture, but not nuclear membranes, may also be penetrated by asbestos fibres (Richards *et al.*, 1974; Henderson *et al.*, 1975). In addition, studies with macrophages have demonstrated that lysosomal membranes may be damaged by asbestos fibres (Parazzi *et al.*, 1968; Allison, 1969). Despite certain aspects common to both silica and asbestos fibres in their interaction with cellular components, there are differences which preclude generalisations about the mechanism of action of mineral dusts (Allison, 1971). For example, the lack of appreciable antihaemolytic effect of PVPNO in asbestos haemolysis implies that the mechanism of haemolysis and the mode of its inhibition may differ for silica and chrysotile (Macnab and Harington, 1967). In studying the

action of PVPNO on the cytopathic effects of chrysotile asbestos dust *in vivo* and *in vitro*, Davis (1972) found that the polymer, in contrast to its effectiveness in silicosis, was unable to modify asbestos lesions. Because of the limited information concerning the mechanism by which asbestos fibres specifically interpose in the inductive process of interferon synthesis (Johnston and Burke, 1973), and the diverse current hypotheses regarding the prevention by PVPNO of the adverse biological action of mineral dusts on cell integrity and function (Holt, 1971), it would be premature to try to explain the protection conferred by PVPNO against the activity of asbestos fibres until these questions have been answered.

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References

- Allison, A. C. (1969). The role of lysosomes in the action of drugs and hormones. *Advances in Chemotherapy*, **3**, 253-302.
- Allison, A. C. (1971). Effects of silica and asbestos on cells in culture. In *Inhaled Particles III*, volume 1, pp. 437-445. Edited by W. H. Walton. Unwin Brothers Limited: The Gresham Press, Old Woking, Surrey, England.
- Bairati, A., and Castano, P. (1968). Studio al microscopio elettronico della localizzazione, nel fegato dei topi, di polimeri sintetici citoprotettivi nei confronti della silice. *La Medicina del Lavoro*, **59**, 81-104.
- Beck, E. G. (1970). Die Reaktion in vitro gezüchteter Zellen auf partikelförmige Luftverunreinigungen und hochpolymere Stoffe. *Forschungsberichte des Landes Nordrhein-Westfalen*, No. 2083, Westdeutscher Verlag: Köln und Opladen.
- Beck, E. G., and Boje, H. (1967). Zytologische Untersuchungen über die Wirkung von Poly 2-vinyl-pyridine N-oxide in der Zellkultur. In *Fortschritte der Staubbungenforschung*, volume 2, pp. 231-236. Edited by H. Reploh and H. J. Einbrodt. Niederrheinische Druckerei: Dinslaken.
- Beck, E. G., Bruch, J., and Brockhaus, A. (1963). The influence of polyvinylpyridine-N-oxide (P204) on the cytotoxic action of quartz on mouse fibroblasts (Strain L). *Zeitschrift für Zellforschung*, **59**, 568-576.
- Bruch, J. (1967). Submikroskopische Beobachtungen nach kurzzeitiger Applikation von Poly-2-vinylpyridin-N-oxid. In *Fortschritte der Staubbungenforschung*, volume 2, pp. 273-276. Edited by H. Reploh and H. J. Einbrodt. Niederrheinische Druckerei: Dinslaken.
- Comolli, R. (1967). Cytotoxicity of silica and liberation of lysosomal enzymes. *Journal of Pathology and Bacteriology*, **93**, 241-253.
- Davis, J. M. G. (1972). The effects of polyvinyl pyridine-N-oxide (P204) on the cytopathogenic action of chrysotile asbestos *in vivo* and *in vitro*. *British Journal of Experimental Pathology*, **53**, 652-658.
- Gainer, J. H. (1972a). Effects of arsenicals on interferon formation and action. *American Journal of Veterinary Research*, **33**, 2579-2586.
- Gainer, J. H. (1972b). Increased mortality in encephalomyocarditis virus-infected mice consuming cobalt sulfate: Tissue concentrations of cobalt. *American Journal of Veterinary Research*, **33**, 2067-2073.
- Grundmann, E. (1967). Experimentelle Untersuchungen über die zelluläre Speicherung des Polyvinylpyridin-N-oxids. In *Fortschritte der Staubbungenforschung*, volume 2, pp. 223-229. Edited by H. Reploh and H. J. Einbrodt. Niederrheinische Druckerei: Dinslaken.
- Hahon, N. (1974). Depression of viral interferon induction in cell monolayers by coal dust. *British Journal of Industrial Medicine*, **31**, 201-208.
- Hahon, N. (1976). Counteraction of Poly(4-vinylpyridine-N-oxide) on the depression of viral interferon induction by coal dust. *Infection and Immunity*, **13**, 1334-1342.
- Hahon, N., Booth, J. A., and Eckert, H. L. (1973). Cell attachment and penetration by influenza virus. *Infection and Immunity*, **7**, 341-351.
- Hahon, N., Booth, J. A., and Eckert, H. L. (1975). Interferon assessment by the immunofluorescent, immunoperoxidase and hemadsorption cell-counting techniques. *Archives of Virology*, **48**, 239-243.
- Hahon, N., and Eckert, H. L. (1976). Depression of viral interferon induction in cell monolayers by asbestos fibers. *Environmental Research*, **11**, 52-65.
- Harington, J. S., Allison, A. C., and Badami, D. V. (1975). Mineral fibers: Chemical, physicochemical and biological properties. *Advances in Pharmacology and Chemotherapy*, **12**, 291-402.
- Harington, J. S., Miller, K., and Macnab, G. (1971). Hemolysis by asbestos. *Environmental Research*, **4**, 95-117.
- Henderson, W. J., Blundell, G., Richards, R., Hext, P. M., Volcani, B. E., and Griffiths, K. (1975). Ingestion of talc particles by cultured lung fibroblasts. *Environmental Research*, **9**, 173-178.
- Holt, P. F. (1971). Poly(vinylpyridine oxides) in pneumoconiosis research. *British Journal of Industrial Medicine*, **28**, 72-77.
- Johnston, M. D., and Burke, D. C. (1973). Interferon induction by viruses: Molecular requirements. In *Selective Inhibitors of Viral Functions*, pp. 123-148. Edited by W. A. Carter. C.R.C. Press: Cleveland, Ohio.
- Kaw, J. L., Beck, E. G., and Bruch, J. (1975). Studies of quartz cytotoxicity on peritoneal macrophages of guinea pigs pretreated with polyvinylpyridine-N-oxide. *Environmental Research*, **9**, 313-320.
- Lockart, R. Z. (1966). Biological properties of interferons: criteria for acceptance of a viral inhibitor as an interferon. In *Interferons*, pp. 1-20. Edited by N. B. Finter. North-Holland Publishing Co.: Amsterdam.
- Macnab, G., and Harington, J. S. (1967). Haemolytic activity of asbestos and other mineral dusts. *Nature*, **214**, 522-523.
- Nash, T., Allison, A. C., and Harington, J. S. (1966). Physicochemical properties of silica in relation to its toxicity. *Nature*, **210**, 259-261.
- Parazzi, E., Pernis, B., Secchi, G. C., and Vigliani, E. C. (1968). Studies of *in vitro* cytotoxicity of asbestos dust. *La Medicina del Lavoro*, **59**, 561-576.
- Richards, R. J., Hext, P. M., Blundell, G., Henderson, W. J., and Volcani, B. E. (1974). Ultrastructural changes in lung fibroblast cultures exposed to chrysotile asbestos. *British Journal of Experimental Pathology*, **55**, 275-281.
- Schlipköter, H. W., and Brockhaus, A. (1961). Die Hemmung der experimentellen Silikose durch subcutane Verabreichung von Polyvinylpyridin-N-Oxyd. *Klinische Wochenschrift*, **39**, 1182-1188.
- Schnitzer, R. J. (1974). Modification of biological surface activity of particles. *Environmental Health Perspectives*, **9**, 261-266.
- Schnitzer, R. J., Bunescu, G., and Baden, V. (1971). Interaction of mineral fiber surfaces with cells *in vitro*. *Annals of the New York Academy of Sciences*, **172**, 759-772.