ENVIRONMENTAL RESEARCH 52, 83–98 (1990)

Coinhibition of Viral Interferon Induction by Benzo[a]pyrene in Association with Occupation-Related Particles[1]

NICHOLAS HAHON,*,† JAMES A. BOOTH,* AND LYNN FLOWERS‡

*National Institute for Occupational Safety and Health, ALOSH, 944 Chestnut Ridge Road, and †Department of Pediatrics, West Virginia University School of Medicine, Morgantown, West Virginia 26505, and ‡Department of Pharmacology, University of Pennsylvania, 36th Street and Hamilton Walk, Philadelphia, Pennsylvania 19104

Received October 24, 1989

Benzo[a]pyrene (B[a]P) in combination with coal, asbestos, silicate, or metal particles was studied for its inhibitory effects on interferon-α/β induction by influenza virus in rhesus monkey kidney (LLC-MK_2) cell monolayers. B[a]P per se had no adverse effect on the induction process. However, when cell cultures were pretreated with B[a]P that was bioactivated by rat liver S9 homogenate, from 52 to 65% inhibition of interferon induction occurred. Significantly greater ($P < 0.05$) depression (coinhibition) of viral interferon induction (>83%) resulted when bioactivated B[a]P was incorporated with coal particles representative of coal rank (anthracite, bituminous, lignite, peat). Coinhibition affected by bioactivated B[a]P was coal rank-independent but any interferon inhibitory activity affected by coal particles per se was coal rank-dependent. When metals (aluminum, aluminum oxide, ferric oxide, nickel, or chromium) or asbestos fibers (chrysotile, crocidolite, anthophyllite, or amosite) were individually mixed with bioactivated B[a]P, coinhibition of cellular interferon synthesis also resulted which was significantly greater ($P < 0.05$) than that manifested by bioactivated B[a]P or particles per se. Coinhibition of interferon induction by silicates (Min-U-Sil, DQ-12, hypersthene, or wollastonite) and the bioactivated hydrocarbon was not in evidence although some silicates alone partially inhibited the induction process. Viral interferon induction was inhibited in a dose–response manner by B[a]P (±S9) in combination with selected particles. The adsorption of B[a]P to all types of particles was no more than 5.98 μg B[a]P/mg of particles and, moreover, less than 0.5% by weight. These findings provide further evidence that bioactivated B[a]P and occupation-related particles act together to coinhibit a biological defense mechanism, the interferon induction phase of the interferon system. © 1990 Academic Press, Inc.

INTRODUCTION

Benzo[a]pyrene (B[a]P) is representative of a class of compounds, the polycyclic aromatic hydrocarbons (PAH). Because it is a widespread environmental pollutant with demonstrable mutacarcinogenic activity (Gelboin and Ts'o, 1978; Jones and Leber, 1979; Gelboin, 1980), B[a]P has been the focus of both increased public health concern and intensive research. That chemical carcinogens and particles often interact in a synergistic manner to affect biologic systems adversely, or act in tandem as cocarcinogens, compounds the disease potential of these reactants and confounds our understanding of the involved mechanisms.

[1] Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

0013-9351/90 $3.00
Copyright © 1990 by Academic Press, Inc.
All rights of reproduction in any form reserved.

Enhanced carcinogenicity of B[*a*]P in the presence of particles, e.g., asbestos, metal oxides, hematite, and varied forms of carbon, has been reported (Saffiotti *et al.*, 1968; Stenback *et al.*, 1976; Lakowicz and Hylden, 1978; Mossman and Craighead, 1979; Lakowicz and Bevan, 1979; Hart *et al.*, 1980). In terms of documented human health effects, the interaction between asbestos and B[*a*]P, a major PAH component present in cigarette smoke, may account for a majority of asbestos-related cancers (Selikoff *et al.*, 1968; Hammond and Selikoff, 1973; McMillan *et al.*, 1980). Similar synergistic activities involving PAH and occupation-related particles may prevail among workers in coke oven, smelting, and mining operations (Hoffman and Wynder, 1976). Cognizance of these circumstances and the need to comprehend the pathogenesis of ensuing diseases has been an impetus for continued investigations on the interactions among extrinsic particles and these hydrocarbons (Mossman *et al.*, 1983; Chang *et al.*, 1983; Leung *et al.*, 1988).

The interferon system, an important component of the host's nonspecific immunologic defense mechanisms, is regarded as a primary protective determinant activated against viral infections. Interferon, whether classed as a cytokine (α, β) or lymphokine (γ), in addition to its antiviral activity possesses the capability of modifying many biologic responses, i.e., anticellular, immunomodulatory, cell differentiation, and hormone/enzyme induction (Taylor-Papadimitriou, 1980), with the totality of its influence on maintaining cell integrity still incomplete. The process of interferon induction is markedly sensitive to and discriminatory for xenobiotic agents either in soluble or in particle forms. Numerous accounts of the inhibitory effect of known carcinogens as well as occupation-related hazardous particles on interferon-α/β induction in mammalian cell cultures have been recorded (DeMaeyer and DeMaeyer-Guignard, 1967; Hahon and Eckert, 1976; Sonnenfeld *et al.*, 1980; Hahon and Ong, 1980; Hahon *et al.*, 1979; Golemboski *et al.*, 1982; Hahon and Booth, 1984, 1987; Hahon, 1985). Recently compiled data indicate an excellent correlation between mutacarcinogenic potential of chemicals and inhibition of interferon-α/β induction (Sonnenfeld, 1986). In contrast, the ability of exogenous interferon to confer antiviral cellular resistance was not impaired by those reagents that inhibit interferon induction (Hahon and Booth, 1984; 1987, 1988; Degre and Holberg-Petersen, 1986). While the association of B[*a*]P with particles has been noted to affect certain biological systems and to enhance carcinogenicity, it was demonstrated only recently that the combination of bioactivated B[*a*]P with chrysotile resulted in coinhibition of interferon induction which exceeded that of either agent alone (Hahon and Booth, 1986b). The analog B[*e*]P, whether alone or exogenously bioactivated (liver S9), neither affected interferon induction nor enhanced chrysotile's inhibition of interferon synthesis. To enlarge our understanding of this circumstance, the association of B[*a*]P with different particle forms common to the environment and occupation settings was investigated further in relation to the interferon defense mechanism.

This report describes the *in vitro* conditions under which coinhibition of viral interferon induction occurred when mammalian cell monolayers were exposed to bioactivated B[*a*]P in conjunction with representative particles of coal, asbestos, metals, and silicates.

MATERIALS AND METHODS

Viruses and Cell Cultures

Virus strains and cell lines used in this study were obtained from the American Type Culture Collection (Rockville, MD). The Ao/PR/8/34 influenza and parainfluenza (Sendai) viruses used for interferon induction and assay, respectively, were prepared from embryonated chicken eggs and assayed for virus infectivity by the immunofluorescent cell-counting technique (Hahon *et al.*, 1973). Rhesus monkey kidney (LLC-MK_2) and Chang human conjunctival (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 cm^2) with Eagle's minimum essential medium fortified with 100× Essential Vitamin Mixture (10 ml/liter), 200 μM solution L-glutamine (10 ml/liter) to which was added sodium bicarbonate (2.2 g/liter) and fetal bovine serum to 10%. Cells were maintained with the aforementioned medium containing 0.5% fetal bovine serum.

Particles and Reagents

UICC asbestos fiber standards, amosite, anthophyllite, crocidolite, and chrysotile were obtained from Duke Scientific Corp. (Palo Alto, CA). Optical microscope evaluations indicated that from 96 to 98% of the asbestos fibers were 30 μm or less in length.

Chrysotile (short) fibers of length and diameter, 2.1 and 0.07 μm, respectively, were obtained from the National Institute of Environmental Health Sciences (Research Triangle Park, NC). The fibers were chemically modified by using the process described by Flowers (1982). Briefly, this involved adding asbestos fibers to an aqueous metallic salt solution, preferably ammonium ferric sulfate ($NH_4Fe(SO_4)_2$) that had been heated to 100°C, and then agitating the slurry for 10 min. After cooling, the hydrated asbestos fibers were collected on a Whatman No. 1 filter by vacuum filtration, dried at 100°C for 16 hr, and then resuspended by agitation in a blender. The process formed a new asbestos fiber surface characterized as a "metal-micelle" polymer.

Metal particles were obtained from Alpha Products (Danners, MA) and their designated mean diameters were chromium (1–4 μm) and nickel (1 μm). Others that were obtained from Duke Scientific Corp. (Palo Alto, CA) were aluminum oxide (1–4 μm), ferric oxide (5–10 μm), and aluminum (0.3–1.2 μm).

European and American silicate standards, DQ-12 (Dörentrup quartz) and Min-U-Sil (pulverized Oriskany sandstone), were gifts from Dr. I. M. Reisner (Essen, West Germany) and Pennsylvania Sand and Glass Corp. (Pittsburgh, PA), respectively. Hypersthene and wollastonite were obtained from Geoscience Resources (Burlington, NC). Silicate minerals were ground in a Microjet 5 Spectomill (Micro Materials Corp., Westbury, NY) to a size of approximately 1.0 μm in diameter as determined with a Coulter Counter (Coulter Instruments, Model ZB, Hialeah, FL).

Coal particles tested, their origin, and respective mean diameters (μm), as determined by a Coulter Counter, were anthracite, Central Pennsylvania (2.55);

bituminous, Pittsburgh, Pennsylvania (2.52); lignite, Texas (2.57); and peat, North Carolina (2.60). Suspensions of coal dust and all other test particles (w/v) were prepared in phosphate-buffered saline (PBS), pH 7.1, and sterilized in an autoclave at pressure of 20 lb/in.^{2}, 126°C, for 15 min.

B[*a*]P obtained from Sigma Chemical Co. (St. Louis, MO) was dissolved in dimethyl sulfoxide (DMSO) 10 mg/ml, to make a stock preparation of 3.96 μmol/ml. Desired test concentrations were prepared in maintenance medium and passed through Millex GS (0.22 μm) filters (Millipore Corp., Bedford, MA) to ensure sterility. The highest final concentration of DMSO used in experimental tests was 1.0% which was neither detrimental to cell viability nor detrimental to interferon induction.

Liver homogenate (9000*g*) supernatant fraction (S9) was prepared from the livers of male Wistar/Lewis rats (225 g/rat) after induction with Aroclor 1254 (ip 500 mg/kg body wt) as described by Ames *et al.* (1975). For experimental use, 0.5% suspension of S9 homogenate was prepared in maintenance medium and then passed through a 0.45-μm Nalge Filter Unit (Nalge Co., Rochester, NY) to obtain sterile preparations. The suspension, after filtration, contained 77.5 μg of protein/ml (Lowry *et al.*, 1951).

Anti-human IFN-α and IFN-β were obtained from Nutritional Biochemicals (Cleveland, OH).

Interferon induction. Duplicate experiments were performed, and the general procedure used to study the effect of different combinations of particles and B[*a*]P on viral interferon induction was carried out as follows: mixtures consisting of 0.05- to 0.5-mg particles in conjunction with 3.96 nmol of B[*a*]P in either a 10-ml volume of maintenance medium or a 0.5% S9 suspension in maintenance medium were shaken at 35°C for 1 hr. The mixtures were added to 75-cm^2 plastic flasks containing complete LLC-MK$_2$ cell monolayers (2×10^7 cells) which were then incubated at 35°C for 24 hr. Residual medium was decanted and 2 ml of influenza virus, which had been inactivated by ultraviolet irradiation for 45 sec at a distance of 76.2 mm and wavelength of 253.7 nm, was added onto cell monolayers that were then incubated at 35°C for 2 hr. The multiplicity of infection was approximately 2.0. Inoculum was removed and 10 ml of maintenance medium was added to each flask. After incubation at 35°C for 24 hr, supernatant fluid was decanted and centrifuged at 100,000*g* for 1 hr and dialyzed against HCl-KCl buffer, pH 2.0, at 4°C for 24 hr. Dialysis was continued against two changes of PBS, pH 7.1, at 4°C for 24 hr. Fluids were passed through Millex filters GV (0.22 μm; Millipore Corp., Bedford, MA) to obtain sterile preparations. Samples were stored at −80°C until they were assayed for interferon activity. Preparations with antiviral activity possessed the biological and physical properties ascribed to viral interferons (Lockart, 1973; Stewart, 1981). Controls consisting of cell monolayers which were not treated with either particles or B[*a*]P were handled exactly as described above. Viral-induced interferon from primate cell cultures contained both interferon-α and interferon-β as determined by interferon-neutralization tests (WHO, 1984).

Interferon assay. An immunofluorescent cell-counting assay of interferon that had been described previously was used to determine the interferon potency of

test samples (Hahon, 1981). Interferon-treated 1-5c-4 cell monolayers were challenged with 10^4 cell-counting units of Sendai virus, and infected cells were visualized by 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, 0.89 interferon unit corresponded to 1.0 unit of National Institutes of Health reference standard Hu IFNβ (G-023-902-527). A 50% or greater decrease of interferon induction, which exceeds 95% confidence limits of the assay, was considered significant (Hahon, 1981).

Adsorption of B[a]P to Particles

To determine the quantity of B[*a*]P adsorbed to particles, B[*a*]P (3.96 μmole) in a 1.0-ml volume was added to 5-mg amounts of particles suspended in a 0.5-ml volume of maintenance medium which was then agitated at 35°C for 1 hr. Thereafter, the mixture was centrifuged at 2000*g* for 15 min, the supernatant fluid was discarded, and the sedimented particles were washed with 10 ml water. The above washing sequence was repeated and, after the final centrifugation, sedimented particles were dispersed in 2 ml acetone with agitation at 22°C for 1 hr. The mixture was then filtered (Millex FH, 0.22 μm; Millipore) and the fluid portion was either assayed for B[*a*]P content or stored at −20°C for subsequent assessment.

The analysis of B[*a*]P was carried out using a high-pressure fluid liquid chromatography (HPLC) method similar to that described by Selkirk *et al.* (1974). The HPLC system (Waters Associated, Milford, MA) was equipped with two Model 6000A pumps, a Model 710B automatic sample injector, a Model 730 data nodule, a Model 720 system controller, a Model 440 absorbance detector, and a Z-module radial compression system. Compounds were separated with a C_{18} radial-pak cartridge (10 μm) using a 30-min linear gradient of 65–100% methanol–water mixture. The solvent flow rate was 2 ml/min, and the chromatographic system was operated at ambient temperature (21–23°C). The eluant was monitored by absorbance at 254 nm and the ultraviolet detector was operated at a sensitivity of 0.05 absorbance unit full scale. For quantitation of B[*a*]P, the retention times and peak areas of extracted products were compared to those of authentic B[*a*]P.

Statistics

Student's *t* test and analysis of variance were used to determine statistical significance.

RESULTS

The cytocidal effect of different concentrations of B[*a*]P and particles, individually and in combination, were determined because cell viability is a prerequisite for interferon induction/production with the designated quantities of reagents used in experiments. LLC-MK_2 monolayers of nondividing cells (2×10^7) showed no appreciable loss of viability (<5%) at 35°C for 24 hr. When cell monolayers were pretreated with bioactivated B[*a*]P in combination with coal particles, viral interferon induction was significantly ($P < 0.05$) inhibited (Table 1). In the presence of bioactivated B[*a*]P, coal dust of lowest rank (peat) was comparable to that of

TABLE 1
COINHIBITION BY BENZO[*a*]PYRENE AND COAL DUST ON INTERFERON (IFN) INDUCTION BY INFLUENZA VIRUS

B[*a*]P (nmole)	Coal dust (mg)	S9[a] (0.5%)	IFN[b] ($ICDD_{50}$)	IFN inhibition[c] (% ± SEM)
	Anthracite			
3.96	0.5	−	473	68.5 ± 4.8
3.96	0.5	+	180	88.0 ± 2.7[d]
—	0.5	−	494	67.1 ± 2.3
—	0.5	+	524	65.1 ± 3.1
	Bituminous			
3.96	0.5	−	624	58.4 ± 4.9
3.96	0.5	+	188	87.5 ± 1.1[d]
—	0.5	−	545	63.7 ± 2.3
—	0.5	+	557	62.9 ± 3.8
	Lignite			
3.96	0.5	−	1037	30.9 ± 0.2
3.96	0.5	+	185	87.7 ± 2.3[d]
—	0.5	−	1010	32.7 ± 1.3
—	0.5	+	992	33.9 ± 3.2
	Peat			
3.96	0.5	−	1500	0.0 ± 0.0
3.96	0.5	+	243	83.8 ± 2.1[d]
—	0.5	−	1473	1.8 ± 0.9
—	0.5	+	1415	5.7 ± 4.3
	Controls			
3.96	—	−	1389	7.4 ± 3.9
3.96	—	+	506	66.3 ± 2.6
—	—	−	1500	0.0
—	—	+	1500	0.0

[a] Rat liver homogenate 9000*g* supernatant fraction.
[b] Mean IFN reciprocal value of two determinations of 50% infected cell-depressing dilution/10 ml medium from LLC-MK_2 monolayers (~2×10^7 cells).
[c] (Reciprocal IFN $ICDD_{50}$/reciprocal control IFN $ICDD_{50}$)/ − 1.0 × 100.
[d] Student's *t* significance level ($P < 0.05$) relative to B[*a*]P control and coal dust +S9 and −S9.

highest rank coal (anthracite) in depressing interferon induction by 83% or more. This coinhibition of interferon induction by bioactivated B[*a*]P and coal particles was independent of coal rank and was greater than that manifested by either B[*a*]P or coal particles per se regardless of S9 presence or absence. Whereas B[*a*]P had no detrimental effect on interferon induction without bioactivation, the presence of S9 did not increase the inhibitory activity of coal particles on interferon induction. The magnitude of interferon induction inhibited by coal particles per se was dependent on coal rank.

Bioactivated B[*a*]P in combination with any of the five metals and oxides coinhibited interferon induction from 67.9 to 84.2% (Table 2). This exceeded significantly ($P < 0.05$) the inhibitory activity on interferon induction by either B[*a*]P or metal particles per se regardless of S9 presence or absence. Nickel, aluminum, and aluminum oxide particles alone, with or without S9 incorporation, compara-

TABLE 2
COINHIBITION BY BENZO[*a*]PYRENE AND METALLIC PARTICLES ON INTERFERON (IFN) INDUCTION BY INFLUENZA VIRUS

B[*a*]P (nmole)	Metal (mg)	S9[a] (0.5%)	IFN[b] ($ICDD_{50}$)	IFN inhibition[c] (% ± SEM)
	Ferric oxide			
3.96	0.5	−	1568	1.1 ± 0.4
3.96	0.5	+	509	67.9 ± 0.9[d]
—	0.5	−	1560	1.6 ± 1.6
—	0.5	+	1525	3.8 ± 0.7
	Nickel			
3.96	0.5	−	703	55.7 ± 6.1
3.96	0.5	+	272	82.9 ± 1.1[d]
—	0.5	−	791	50.1 ± 10.7
—	0.5	+	666	51.7 ± 8.2
	Aluminum			
3.96	0.2	−	867	45.3 ± 2.6
3.96	0.2	+	394	75.2 ± 5.6[d]
—	0.2	−	896	43.5 ± 4.3
—	0.2	+	918	42.1 ± 1.9
	Aluminum oxide			
3.96	0.2	−	744	53.1 ± 2.1
3.96	0.2	+	251	84.2 ± 1.6[d]
—	0.2	−	704	55.6 ± 3.6
—	0.2	+	720	54.6 ± 2.1
	Chromium			
3.96	0.2	−	1207	23.9 ± 0.3
3.96	0.2	+	362	77.2 ± 0.5[d]
—	0.2	−	1099	30.7 ± 0.6
—	0.2	+	1094	31.0 ± 1.0
	Controls			
3.96	—	−	1578	0.5 ± 0.5
3.96	—	+	747	52.9 ± 7.0
—	—	−	1585	0.0
—	—	+	1585	0.0

[a] Rat liver homogenate 9000*g* supernatant fraction.

[b] Mean IFN reciprocal value of two determinations of 50% infected cell-depressing dilution/10 ml medium from LLC-MK_2 monolayers (~2×10^7 cells).

[c] (Reciprocal IFN $ICDD_{50}$/reciprocal control IFN $ICDD_{50}$)/ − 1.0 × 100.

[d] Student's *t* significance level ($P < 0.05$) relative to B[*a*]P + S9 control and metals + S9 and − S9.

bly and markedly inhibited interferon induction. Chromium and ferric oxide under similar circumstances only moderately or minimally affected the induction process, the former a reflection of dosage (Hahon and Booth, 1984).

Coinhibition of viral interferon induction occurred when bioactivated B[*a*]P was mixed with asbestos fibers of anthophyllite, crocidolite, chrysotile, or amosite (Table 3). The inhibition of interferon induction (>80%) was significantly ($P < 0.05$) greater than that of bioactivated B[*a*]P or that of asbestos fibers per se. Modified chrysotile fibers did not depress interferon induction. In the presence of bioactivated B[*a*]P, these fibers were still capable of significantly inhibiting the interferon induction process in semblance of coinhibition.

TABLE 3
COINHIBITION BY BENZO[*a*]PYRENE AND ASBESTOS ON INTERFERON INDUCTION BY INFLUENZA VIRUS

B[*a*]P (nmole)	Asbestos (mg)	S9[a] (0.5%)	IFN[b] ($ICDD_{50}$)	IFN inhibition[c] (% ± SEM)
	Anthophyllite			
3.96	0.05	−	540	52.7 ± 1.8
3.96	0.05	+	160	86.0 ± 3.3[d]
—	0.05	−	529	53.6 ± 1.8
—	0.05	+	540	52.7 ± 3.9
	Crocidolite			
3.96	0.05	−	528	53.7 ± 0.1
3.96	0.05	+	179	84.3 ± 0.2[d]
—	0.05	−	526	53.9 ± 2.2
—	0.05	+	567	50.3 ± 4.0
	Chrysotile			
3.96	0.05	−	675	40.8 ± 6.6
3.96	0.05	+	203	82.2 ± 1.5[d]
—	0.05	−	721	36.8 ± 1.6
—	0.05	+	677	40.7 ± 0.1
	Modified chrysotile[e]			
3.96	0.05	−	1104	3.2 ± 3.2
3.96	0.05	+	222	80.6 ± 0.6[d]
—	0.05	−	1127	1.2 ± 1.2
—	0.05	+	1104	3.2 ± 3.2
	Amosite			
3.96	0.05	−	806	29.3 ± 8.2
3.96	0.05	+	184	83.9 ± 0.5[d]
—	0.05	−	788	30.9 ± 1.0
—	0.05	+	746	34.6 ± 1.3
	Controls			
3.96	—	−	1054	7.6 ± 3.8
3.96	—	+	399	65.0 ± 2.7
—	—	−	1140	0.0
—	—	+	1140	0.0

[a] Rat liver homogenate 9000*g* supernatant fraction.
[b] Mean interferon reciprocal value of two determinations of 50% infected cell-depressing dilution/10 ml medium from LLC-MK_2 monolayers (~2×10^7 cells).
[c] Reciprocal IFN $ICDD_{50}$/reciprocal control IFN $ICDD_{50}/-1.0 \times 100$.
[d] Student's *t* significance level ($P < 0.05$) relative to B[*a*]P + S9 control and asbestos + S9 and − S9.
[e] Fibers treated with ammonium ferric sulfate at 100°C.

The effect on viral induction of interferon by silicates (DQ-12, Min-U-Sil, hypersthene, and wollastonite) ranged from approximately 50% inhibition (Min-U-Sil) to almost a twofold enhancement of interferon production (wollastonite). Coinhibition of interferon induction was not evident with tested silicates in conjunction with bioactivated B[*a*]P (Table 4). The depression of viral interferon induction by bioactivated B[*a*]P and silicates together was not significantly greater than that of the B[*a*]P (+S9) control.

TABLE 4
ABSENCE OF COINHIBITION BY BENZO[*s*]PYRENE AND SILICATES ON INTERFERON (IFN) INDUCTION BY INFLUENZA VIRUS

B[*a*]P (nmole)	Silicate (mg)	S9[a] (0.5%)	IFN[b] ($ICDD_{50}$)	IFN inhibition[c] (% ± SEM)
	Min-U-Sil			
3.96	0.2	−	550	54.2 ± 0.3
3.96	0.2	+	440	63.4 ± 1.5[d]
—	0.2	−	534	55.5 ± 1.5
—	0.2	+	610	49.2 ± 6.0
	DQ-12 (Dörentrup)			
3.96	0.2	−	880	26.7 ± 1.8
3.96	0.2	+	560	53.4 ± 0.3[d]
—	0.2	−	990	17.5 ± 3.6
—	0.2	+	900	25.0 ± 3.5
	Hypersthene			
3.96	0.2	−	1200	0.0 ± 0.0
3.96	0.2	+	450	62.5 ± 1.6[d]
—	0.2	−	1152	0.4 ± 0.4
—	0.2	+	1092	0.9 ± 0.9
	Wollastonite			
3.96	0.2	−	1920	+1.6 ± 0.1[e]
3.96	0.2	+	470	60.9 ± 2.0[d]
—	0.2	−	1440	+1.7 ± 0.2[e]
—	0.2	+	2160	+1.8 ± 0.2[e]
	Controls			
3.96	—	−	1140	5.6 ± 0.5
3.96	—	+	450	62.5 ± 0.7
—	—	−	1200	0.0
—	—	+	1200	0.0

[a] Rat liver homogenate 9000*g* supernatant fraction.
[b] Mean IFN reciprocal value of two determinations of 50% infected cell-depressing dilution/10 ml medium from LLC-MK_2 monolayers (~2×10^7 cells).
[c] (Reciprocal IFN $ICDD_{50}$/reciprocal control IFN $ICDD_{50}$)/-1.0×100.
[d] Not significant at Student's *t* significance level ($P < 0.05$) relative to B[*a*]P +S9 (control) and Min-U-Sil. Significant difference in relation to all other silicates.
[e] Ratio of interferon increase.

Dose–response relationships between particles, the type selected randomly and representing each of the four particle groups, together with B[*a*]P (±S9), and viral inhibition of viral interferon was determined. The quantity of particles used in tests ranged from 0.01 to 1.0 mg with limitations imposed by the cytotoxicity of mixtures. Results (Fig. 1) show dose-dependent responses between all four types of representative particles (crocidolite, anthracite, Min-U-Sil, and nickel, in combination with B[*a*]P (±S9)) and interferon induction inhibition. All response slopes with test particles approached parallelism. Reduction of interferon induction to 50%, however, required significantly less particles in the presence of bioactivated B[*a*]P than that realized with B[*a*]P (−S9), i.e., sixfold less nickel

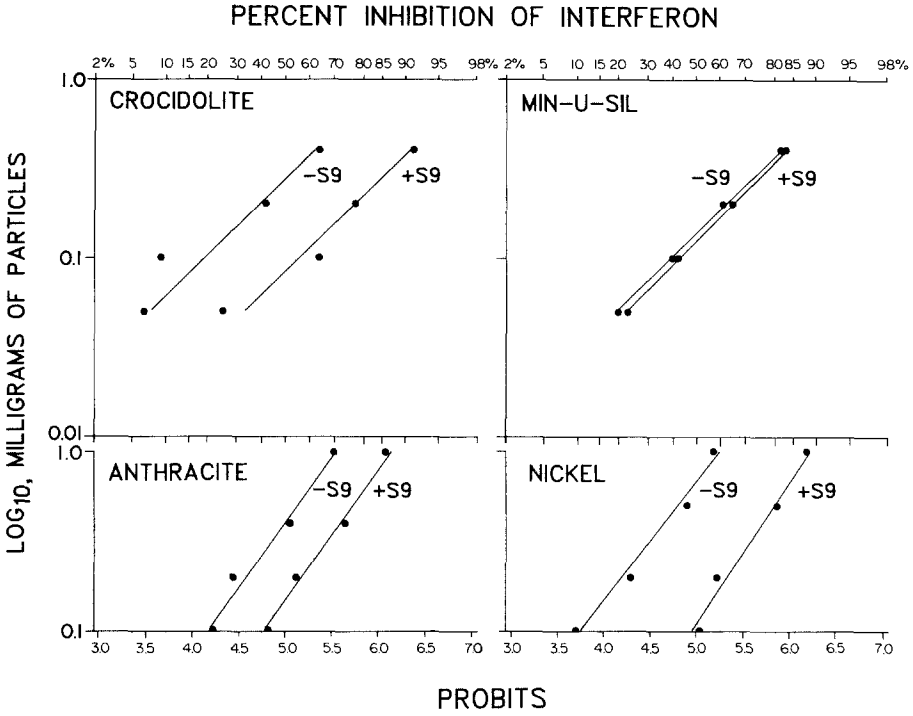

FIG. 1. Dose–response relationship between representative particles, in combination with benzo[*a*]pyrene with and without bioactivation by rat liver S9 homogenate, and inhibition of influenza virus induction of interferon in LLC-MK_2 cell monolayers.

particles, and both threefold less anthracite and crocidolite particles, respectively. The exception was the silicate, Min-U-Sil. The quantity of these particles was equivalent to achieve 50% reduction of interferon synthesis regardless of the state of B[*a*]P.

The adsorption of B[*a*]P to the different particles that comprise the test groups was determined. Results (Table 5) indicate that a range from 1.00 to 5.89 μg B[*a*]P/mg of test particles was adsorbed which was approximately 0.5% or less by weight. Among all the particle groups, however, there was no significant difference in mean values of adsorption, although the silicates appeared to adsorb the lowest quantity of B[*a*]P.

DISCUSSION

The findings of this study affirm that enzymatically activated B[*a*]P in combination with different particles, the majority of which are occupation-related, act together to coinhibit a biologic defense mechanism, the induction phase of the interferon system. The nature of the test particles ranged, in structure, from fibrous to crystalline and, in composition, from organic to metallic with the status of many of the particles previously recognized and characterized in relation to interferon induction inhibition (Hahon and Eckert, 1976; Hahon *et al.*, 1980;

TABLE 5
BENZO[*a*]PYRENE (B[*a*]P) ADSORPTION BY DIFFERENT PARTICLES

Group	Particle[a]	B[*a*]P recovered[b] (μg/100 μl)	Adsorption[c] B[*a*]P/particles (μg/mg)	B[*a*]P[d] adsorbed by wt (% ± SEM)
Coal	Anthracite	1.46	4.38	0.43 ± 0.16[e]
	Bituminous	1.36	4.10	0.41 ± 0.13
	Lignite	1.04	3.13	0.31 ± 0.03
	Peat	0.77	2.32	0.23 ± 0.06
Asbestos	Chrysotile	1.52	4.56	0.45 ± 0.14[e]
	Crocidolite	0.47	1.42	0.14 ± 0.05
	Anthophyllite	0.91	2.74	0.27 ± 0.07
	Amosite	1.15	3.45	0.34 ± 0.12
Metal	Nickel	0.57	1.72	0.17 ± 0.11[e]
	Aluminum	0.72	2.16	0.21 ± 0.11
	Chromium	0.57	1.71	0.17 ± 0.03
	Ferric oxide	1.96	5.89	0.58 ± 0.18
	Aluminum oxide	0.66	2.00	0.20 ± 0.06
Silicate	Min-U-Sil	0.66	2.00	0.20 ± 0.07[e]
	DQ-12	0.48	1.46	0.14 ± 0.05
	Hypersthene	0.33	1.00	0.10 ± 0.04

[a] In a 1.5-ml volume, 1 ml (100 μg) B[*a*]P mixed with 0.5 ml (5 mg) particles at 22°C for 1 hr.
[b] Mean of two determinations, HPLC.
[c] B[*a*]P recovered (μg/100 μl) × 15 (vol)/5 (mg) particles.
[d] (B[*a*]P (adsorbed/μg)/Particles 1000/μg))/× 100.
[e] No significant differences in mean values among groups, $P = 0.14$ by analysis of variance testing.

Hahon, 1983; Hahon and Booth, 1984, 1987). This coinhibition phenomenon prevailed only with bioactivated B[*a*]P in combination with such particles representative of coal, asbestos, metals (exclusive of silicates), and those not normally associated with human health problems (ferrous oxide, peat). B[*a*]P per se had no detrimental effect on the viral induction of interferon in the mammalian cell cultures employed unless the hydrocarbon was bioactivated by exogenous means, i.e., rat liver S9 homogenate (Hahon and Booth, 1986b, 1988).

Coinhibition of interferon induction affected by bioactivated B[*a*]P and coal particles was independent of coal rank whereas any interferon inhibitory activities affected by coal particles per se was coal rank-dependent with the latter confirming previous findings (Hahon, 1983). Maximal inhibition of viral interferon induction was noted by coal particles of high rank and correlated with coal's position in the carboniferous series: anthracite, bituminous, lignite, peat. Whereas all coal particles regardless of rank appeared to have comparable adsorptive capacities for B[*a*]P, the amounts of B[*a*]P adsorbed were less than 0.5% by weight. This fractional but biologically active quantity of B[*a*]P, almost uniformly adsorbed to coal particles regardless of composition or rank, may account for the coal rank independency exhibited in the coinhibition phase of interferon induction.

When cell monolayers were first exposed to bioactivated B[*a*]P in combination with any of the five metals or corresponding compounds, aluminum, aluminum oxide, ferric oxide, chromium, and nickel, coinhibition values significantly ex-

ceeded ($P < 0.05$) those interferon inhibition values of either metals or hydrocarbon alone. The quantity of B[*a*]P adsorbed to metals were small (<0.21% by wt), ferric oxide being the exception (0.58% by wt). Nevertheless, these minute amounts of B[*a*]P in combination with metals was sufficient to increase the overall inhibitory activity of the hydrocarbon on the depression of interferon induction. Employing another criterion, Hubbard *et al.* (1986) reported that an increase in mutagenic activity results when B[*a*]P is adsorbed to alumina, titanium dioxide, or ferric oxide. Heavy metals and hydrocarbons simultaneously introduced to test hosts have been also noted to act as cocarcinogens or to increase the *in vivo* concentration of metals (Saffiotti *et al.*, 1968; Stenback *et al.*, 1976; Lakowicz and Bevan, 1979; Fair and Fortner, 1987).

In numerous studies involving B[*a*]P and asbestos fibers, synergistic or augmentation effects have been reported relative to cell transformation-oncogenic activity (Poole *et al.*, 1983), tumor production (Miller *et al.*, 1965; Pylev and Shabad, 1973; Topping and Nettesheim, 1980; Mossman and Craighead, 1981; Craighead and Mossman, 1982), mutagenicity (Reiss *et al.*, 1983), and B[*a*]P uptake, metabolism, and binding to microsomes and nucleic acids (Lakowicz and Bevan, 1979; Lakowicz *et al.*, 1980; Kandaswami and O'Brien, 1980; Brown *et al.*, 1983; Chang *et al.*, 1983). A semblence of coinhibition of viral interferon induction was demonstrated, *in vitro,* only recently, when bioactivated B[*a*]P was in contact with chrysotile asbestos fibers (Hahon and Booth, 1986b). This phenomenon of coinhibition was expanded by this current study when bioactivated B[*a*]P was mixed with chrysotile, anthophyllite, crocidolite, or amosite. Modified chrysotile per se, in contrast to the ability of other asbestos fibers, had no detrimental influence on the interferon induction phase (Hahon *et al.*, 1986). Nevertheless, these modified "innocuous" chrysotile fibers, in combination with bioactivated B[*a*]P, were capable of promoting the activity of B[*a*]P resulting in the depression of viral interferon induction in a coinhibition manner. The chemical modification of chrysotile fibers, apparently, did not impede events associated with microsomal metabolism of B[*a*]P, transfer, or binding of activated metabolites to macromolecular elements of target cells.

Coinhibition of viral interferon induction was not in evidence when bioactivated B[*a*]P was mixed with silicates: DQ-12, Min-U-Sil, hypersthene, or wollastonite. The former two silicates alone partially depressed intereferon induction in combination with bioactivated B[*a*]P but the magnitude of inhibition was not significantly greater than that of the B[*a*]P control alone or with S9. In contrast, wollastonite, in common with other silicates of the pyroxenoid group, enhanced interferon production (Hahon and Booth, 1987) but not in the presence of bioactivated B[*a*]P. Although the amount of B[*a*]P adsorbed by silicates was no more than 0.2% by weight and not significantly different from that adsorbed by other tested particles, i.e., asbestos, metals, and coal, for some undetermined reason, coinhibition of interferon induction was not evident with any of the tested silicates. The quantity of B[*a*]P adsorbed by silicates and that by other particles used in this study was generally in agreement with the amount of 0.3% by weight noted with other particles (Lakowicz *et al.*, 1980). Using fluorescence spectroscopic methods and model vesicles, Lakowicz and Bevan (1979) and Lakowicz *et al.*

(1980) reported that silica can enhance microsomal availability and increase the rate of membrane uptake of B[*a*]P. In deference to these observations, this does not preclude the possibility, in considering the biologic activity standard employed in our current study, that the absence of a coinhibition effect on interferon induction may be related to the effect of silicates on microsomal enzymes resulting in partial metabolism of B[*a*]P and the formation of biologic unreactive metabolites, or resistance of metabolites to desorption from silicates. Pawlak (1982) documented the numerous conditions that may affect B[*a*]P activation in mammalian cells and resultant metabolite formation in relation to monooxygenases, enzymes prominant in B[*a*]P metabolism. The type of enzyme inducer, enzyme inhibitors, and modulators of metabolism, e.g., phenols and asbestos, are all modifying factors that can influence the type and levels of monooxygenases generated.

Although it has been demonstrated that B[*a*]P metabolites, phenols, dihydrodiols, and diol epoxides I and II, are active inhibitors of viral interferon induction (Hahon and Booth, 1986a), it is not presently known which of these metabolites are formed when B[*a*]P is bioactivated in the presence of different particles or, for that matter, the mechanisms by which either particles or metabolites per se and in combination act to affect adversely the induction phase of the interferon system. There are indications that particles, i.e., asbestos, can shift the metabolism of B[*a*]P to generate metabolites having a more carcinogenic profile (Eneanya *et al.*, 1979). Studies are in progress to determine the inhibitory activity of major B[*a*]P metabolites combined with particles on interferon induction. The extensive influence of the interferon system on biologic responses is well known (Taylor-Papadimitriou, 1980, 1985; DeMaeyer and DeMaeyer-Guignard, 1988); therefore, it seems plausible that alterations of this cellular defense mechanism may play a role in the inchoate activities associated with B[*a*]P-particle cocarcinogenesis. The sensitivity and discriminatory ability of the interferon induction phase to inhibition by xenobiotics, either in particle or in soluble forms and in combination, provides an augmentative means for assessing potential metabolic-dependent carcinogenic combinations of hydrocarbons and particles.

ACKNOWLEDGMENT

We thank Dr. Martin Petersen for his assistance in the statistical analysis of data.

REFERENCES

Ames, B. N., McCann, J., and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity tests. *Mutat. Res.* **31**, 347–365.

Brown, R. C., Fleming, G. T. H., and Knight, A. I. (1983). Asbestos affects the in vitro uptake and detoxification of aromatic compounds. *Environ. Health Perspect.* **51**, 315–318.

Chang, M. J. W., Singh, N. P., and Hart, L. W. (1983). Effects of chrysotile and co-exposure on BaP binding in normal fibroblasts. *Environ. Health Perspect.* **51**, 241–244.

Craighead, J. E., and Mossman, B. T. (1982). The pathogenesis of asbestos-associated diseases. *N. Engl. J. Med.* **24**, 1446–1455.

Degre, M., and Holberg-Petersen, M. (1986). Influence of carcinogenic agent, 7.12 dimethylbenz(α) anthracene (DMBA), on production of interferon alpha/beta in murine and human cells. *Acta Pathol. Microbiol. Immunol. Scand. Sect. B* **94**, 51–55.

DeMaeyer, E., and DeMaeyer-Guignard, J. (1967). Effect of different carcinogenic agents on the

production of interferon in tissue culture and in the animal. *In* "Interferon" (G. E. W. Wolstenholme and M. O'Connor, Eds.), pp. 218–229. Little, Brown, Boston.

DeMaeyer, E., and DeMaeyer-Guignard, J. (1988). "Interferons and Other Regulatory Cytokines." Wiley, New York.

Eneanya, D. I., Daniel, R., and Hart, R. W. (1979). Effect of asbestos (chrystole intermediates) on the metabolism of benzo(*a*)pyrene in Syrian hamster embryo cells. *Fed. Proc.* **39**, 4027–4033.

Fair, P. H., and Fortner, A. R. (1987). Effect of ingested benzo[*a*]pyrene and cadmium on tissue accumulation, hydroxylase activity, and intestinal morphology of the black sea bass, *Centropsistis* striata. *Environ. Res.* **42**, 185–195.

Flowers, E. S. (1982). Metal-micelle asbestos and treatment of asbestos and other mineral silicates to reduce their harmful properties. U.S. Patent 4,328,197, May 4, assigned to Flow General, Inc., Mclean, Va.

Gelboin, H. V. (1980). Benzo[*a*]pyrene metabolism, activation, and carcinogenesis: Role and regulation of mixed-function oxidases and related enzymes. *Physiol Rev.* **60**, 1107–1166.

Gelboin, H. V., and Ts'o, P. O. P. (1978). "Polycyclic Hydrocarbons and Cancer," Vols. 1 and 2. Academic Press, New York.

Golemboski, K. A., Delor, D. K., Streips, U. N., and Sonnenfeld, G. (1982). Effect of 7,12-dimethylbenzo(*a*)anthracene on production and action of gamma interferon. *J. Natl. Cancer Inst.* **68**, 993–996.

Hahon, N. (1981). Hemadsorption and fluorescence determinations for assay of virus-yield induction of interferon. *In* "Methods in Enzymology" (S. Pestka, Ed.), Vol. 78, Part A, pp. 373–381. Academic Press, New York.

Hahon, N. (1983). Effect of coal rank on the interferon system. *Environ. Res.* **30**, 72–79.

Hahon, N. (1985). Inhibition of viral interferon induction in mammalian cell cultures by azo dyes and derivatives activated with rat liver S9 fraction. *Environ. Res.* **37**, 228–238.

Hahon, N., and Booth, J. A. (1984). Effect of chromium and manganese particles on the interferon system. *J. Interferon Res.* **4**, 17–27.

Hahon, N., and Booth, J. A. (1986a). Benzo[a]pyrene metabolites: Effects on viral interferon induction. *J. Interferon Res.* **6**, 591–602.

Hahon, N., and Booth, J. A. (1986b). Coinhibition of viral interferon induction by benzo[*a*]pyrene and chrysotile asbestos.*Environ. Res.***40**, 103–109.

Hahon, N., and Booth, J. A. (1987). Silicate minerals and the interferon system. *Environ.Res.* **43**, 395–409.

Hahon, N., and Booth, J. A. (1988). Benzo[*a*]pyrene: Kinetics of in vitro bioactivation in relation to inhibition of viral interferon induction. *J. Interferon Res.* **8**, 151–167.

Hahon, N., Booth, J. A., and Eckert, H. L. (1973). Cell attachment and penetration by influenza virus. *Infect. Immun.* **7**, 341–351.

Hahon, N., Booth, J. A., and Pearson, D. J. (1980). Inhibition of viral interferon induction in mammalian cell monolayers by metallic copper, aluminum, and nickel particles. *In* "The In Vitro Effects of Mineral Dusts" (R. C. Brown, M. Chamberlain, R. Davis, and I. P. Gormby, Eds.), pp. 219–230. Academic Press, New York.

Hahon, N., Booth, J. A., and Stewart, J. D. (1979). Aflatoxin inhibition of viral interferon induction. *Antimicrob. Agents Chemother.* **16**, 227–282.

Hahon, N., and Eckert, H. L. (1976). Depression of viral interferon induction in cell monolayers by asbestos fibers. *Environ. Res.* **11**, 52–65.

Hahon, N., and Ong, T-M. (1980). Action of antischistosomal drugs, hycanthone and its analog IA-4N-oxide, on viral interferon induction. *J. Toxicol. Environ. Health* **6**, 705–712.

Hahon, N., Vallyathan, V., Booth, J. A., and Sepulveda, M. J. (1986). *In vitro* biologic responses to native and surface-modified asbestos. *Environ. Res.* **39**, 345–355.

Hammond, E. C., and Selikoff, I. J. (1973). Relation of cigarette smoking to risk of death of asbestos-associated disease among insulation workers in the United States. *In* "Biological Effects of Asbestos" (P. Bogovski, V. Timbrell, J. C. Gilson, and J. C. Wagner, Eds.), pp.213–317. IARC, Lyon.

Hart, R. N., Daniel, F. B., Kindig, O. R., Beach, C. A., Joseph, L. B., and Wells, R. C. (1980).

Elemental modifications of polycyclic aromatic hydrocarbon metabolism in human fibroblasts. *Environ. Health Perspect.* **34**, 59–68.

Hoffman, D., and Wynder, E. L. (1976). Smoking and occupational cancers. *Prev. Med.* **5**, 245–261.

Hubbard, S. A., Davis, P. J. B., Hunt, C. M., and McDonald, T. (1986). Effects of benzo[*a*] pyrene-coated particles in a bacterial mutagenicity test and on macrophages in culture. *Food Chem. Toxicol.* **24**, 697–698.

Jones, P. N., and Leber, P. (1979). "Polynuclear Aromatic Hydrocarbons. Third International Symposium on Chemistry and Biology-Carcinogenesis and Mutagenesis." Ann Arbor Science Publishers, Inc., Ann Arbor, MI.

Kandaswami, C., and O'Brien, P. J. (1980). Effects of asbestos on membrane transport and metabolism of benzo(*a*)pyrene. *Biochem. Biophys. Res. Commun.* **97**, 794–801.

Lakowicz, J. R., and Bevan, D. R. (1979). Effects of asbestos, iron oxide, silica, and carbon black on the microsomal availability of benzo(*a*)pyrene. *Biochemistry* **18**, 5170–5176.

Lackowicz, J. R., Bevan, D. R., and Riemer, S. C. (1980). Transport of a carcinogen, benzo[*a*]-pyrene, from particulates to lipid bilayers. *Biochim. Biophys. Acta* **629**, 243–258.

Lackowicz, J. R., and Hylden, J. L. (1978). Asbestos-mediated membrane uptake of benzo(*a*)pyrene observed by fluorescence spectroscopy. *Nature (London)* **275**, 446–448.

Leung, H. W., Henderson, R. F., Bond, J. A., Monderly, J. L., and McClellan, R. O. (1988). Studies on the ability of rat lung and liver microsomes to facilitate transfer and metabolism of benzo[*a*]pyrene from diesel particles. *Toxicology* **5**, 1–9.

Lockart, R. Z., Jr. (1973). Criteria for acceptance of a viral inhibitor as an interferon and a general description of the biological properties of known interferons. *In* "Interferons and Interferon Inducers" (N. B. Finter, Ed.), pp. 11–27. North Holland, Amsterdam.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

McMillan, G. H. G., Pethybridge, R. J., and Sheers, G. (1980). Effects of smoking on attach rates of pulmonary and pleural lesions related to exposure to asbestos dusts. *Brit. J. Ind. Med.* **37**, 268–272.

Miller, L., Smith, W. E., and Berliner, S. W. (1965). Tests for effect of asbestos on benzo(*a*)pyrene carcinogenesis in the respiratory tract. *Ann. N.Y. Acad. Sci.* **132**, 489–500.

Mossman, B. T., and Craighead, J. E. (1979). Use of hamster tracheal organ cultures for assessing the carcinogenic effects of inorganic paticulates on the respiratory epithelium. *Prog. Exp. Tumor Res.* **24**, 37–47.

Mossman, B. T., and Craighead, J. E. (1981). Mechanisms of asbestos carcinogenesis. *Environ. Res.* **25**, 269–280.

Mossman, B. T., Eastman, A., Landesman, J. M., and Bresnick, E. (1983). Effects of crocidolite and chrysotile asbestos on cellular uptake and metabolism of benzo[*p*]pyrene in hamster tracheal epithelial cells. *Environ. Health Perspect.* **51**, 331–335.

Pawlak, A. L. (1982). Factors affecting benzo(*a*)pyrene activation mammalian cells. *Arch. Immunol. Ther. Exp.* **30**, 247–255.

Poole, A., Brown, R. C., and Fleming, G. T. A. (1983). Study of the cell transforming ability of amosite and crocidolite asbestos and the ability to induce changes in the metabolism and macromolecular binding of benzo(*a*)pyrene in C3H10T1/2 cells. *Environ. Health Perspect.* **51**, 319–324.

Pylev, L. N., and Shabad, L. M. (1973). Some results of experimental studies in asbestos carcinogenesis. *In* "Biological Effects of Asbestos" (P. Bogovski, V. Timbrell, J. C. Gilson, and J. C. Wagner, Eds.), pp. 312–317. IARC, Lyon.

Reiss, B., Tong, C., Teland, S., and Williams, C. M. (1983). Enhancement of benzo[*a*]pyrene mutagenicity by chrysotile asbestos in rat liver epithelial cells. *Environ. Res.* **31**, 100–104.

Saffiotti, V., Cefis, F., and Kolb, L. H. (1968). A method for the experimental induction of bronchogenic carcinoma. *Environ. Res.* **28**, 104–124.

Selikoff, I. J., Hammond, E. C., and Churg, J. (1968). Asbestos exposure, smoking and neoplasia. *J AMA* **204**, 104–110.

Selkirk, J. K., Croy, R. G., and Gelboin, H. V. (1974). Benzo(*a*)pyrene metabolites: Efficient and rapid seaparation by high-pressure liquid chromatography. *Science* **104**, 169–171.

Sonnenfeld, G. (1986). Interactions of the interferon system with cellular metabolism. *In* "Clinical

Applications of Interferons and their Inducers" (D. A. Stringfellow, Ed.), 2nd ed., pp. 43–59. Dekker, New York.

Sonnenfeld, G., Barnes, M. C., Schooler, J., and Streips, U. N. (1980). Inhibition of interferon induction as a screen for the carcinogenic potential of chemicals. *In* "Interferon: Properties and Chemical Uses" (A. Kahn, N. O.Hill, and G. L. Dorn, Eds.), pp. 589–598. Leland Fikes Foundation Press, Dallas.

Stenback, F., Rowland, J., and Sellakumar, A. (1976). Carcinogenicity of benzo[*a*]pyrene and dusts in hamster lung (instilled intratracheally with titanium, carbon and ferric oxide). *Oncology* **33**, 29–34.

Stewart, W. E., II (1981) Interferons: Their purification and characterization. *In* "The Interferon System" (W. E. Stewart II, Ed.), 2nd ed., pp. 134–183. Springer-Verlag, Wien/New York.

Taylor-Papadimitriou, J. (1980). Effects of interferon on cell growth and function. *In* "Interferon" (I. Gresser, Ed.), Vol. 2, pp. 13–46. Academic Press, New York.

Taylor-Papadimitriou, J. (1985). "Interferons. Their Impact in Biology and Medicine." Oxford Univ. Press, Oxford.

Topping, D. C., and Nettesheim, P. (1980). Two stage carcinogenesis studies with asbestos in Fischer 344 rats. *J. Natl. Cancer Inst.* **65**, 627–630.

WHO Expert Committee (1984). Standardization of interferons. *Antiviral Res.* **14**, 75–98.