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Interferon Induction Inhibition and Mutagenic Activity of Nitrosated Coal Dust Extract

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Specified cytotoxicity and mutagenicity of coal dust extract (mixture of solvent extractions of bituminous coal nitrosated by $NaNO_2$) were investigated because of the association of an excess risk of gastric cancer in coal miners. The effect of nitrosated coal dust on a cellular defense component of the interferon system, the induction of interferon (α/β) in mammalian cell cultures by influenza virus, and mutagenicity, using the *Salmonella*/microsome assay, were determined. Nitrosated coal dust extract contained both bioactivation-independent and -dependent (microsome enzymatic activation) compounds that significantly inhibited the viral induction of interferon by >50%. Nitrosated extract was mutagenic but exhibited no increase in mutagenic activity in the presence of microsomal enzymes. With further extraction of nitrosated coal dust extract by horse serum and fractionation thereof, the soluble chemical complexes formed with fractions of high molecular weight without bioactivation were dominant in both mutagenicity and inhibition of interferon induction. Low-molecular-weight fractions, with or without a metal chelator, and with or without bioactivation, all inhibited interferon induction comparably and significantly. There was no mutagenic activity manifested by these serum fractions. Metal–serum complexes were either not formed, or, if present, were ineffectual according to the biologic criteria employed. The findings of this study are discussed in terms of the association between nitrosated coal dust and gastric carcinogenesis.

INTRODUCTION

While epidemiologic studies in both the United States and the United Kingdom indicate an excess risk to gastric cancer in coal miners than in nonminers (Stocks, 1962; Matolo *et al.*, 1972; Rockette, 1977; Ames, 1983), the association and pathogenesis of the disease have yet to be clearly defined and characterized. Interrelations of worker's lifestyle, duration of exposure to coal dust, rank of coal, and confounding effects with other variables may affect miner's susceptibility to gastric cancer (Ames, 1982). In terms of carcinogenic potential, the heterogeneous nature of coal dust is of concern particularly with respect to its inherent content of polynuclear aromatic hydrocarbons (PNA) and metals (Falk and Jurgelski, 1979). Numerous studies have established the formation of mutacarcinogens from metabolized PNAs and *N*-nitrosamines from the reaction of secondary amines and nitrite under acidic conditions (Sen *et al.*, 1969; Lijinsky *et al.*, 1972; Mirvish, 1975; Whong *et al.*, 1979; Gelboin, 1980). A possible association between nitroso compounds and human gastric cancer has been implied (Mirvish, 1971).

Using the *Salmonella*/microsome assay, Whong *et al.* (1983) showed that nitro-

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sated coal dust extract at low pH was highly mutagenic and independent of metabolic activation. In response to nitrosated coal dust extract, genotoxic activity in mammalian cells was exemplified by increased sister chromatid exchanges, chromosomal aberrations, and gene mutations (Tucker *et al.*, 1984). These findings add credibility to the viewpoint that mutacarcinogenic substances are present in coal dust and pose the possibility that they may be activated in the acidic stomach environment by the interaction of swallowed coal dust and nitrosating agents, i.e., nitrite, an additive in meat, *inter alia* (Whong *et al.*, 1983).

Bocci *et al.* (1984) reported that the lymph from abdominal tissue of healthy rabbits possesses antiviral activity indicative of interferon. However, neither the physiologic role of interferon in gut-associated lymphoid tissue nor the possible effect of nitrosated coal dust extract on interferon levels in abdominal lymph tissue are known. That coal dust per se is capable of inhibiting viral interferon induction in mammalian cells has been noted previously (Hahon, 1974, 1983). Although antiviral activity is a common attribute, interferons may modulate numerous aspects of the immune system, stimulate or inhibit intracellular enzymes, and influence the proliferation or inhibition of both normal and malignant cells (Taylor-Papadimitriou, 1980). The sensitive nature of the interferon induction process has provided a credible means for evaluating the insidious potential of particulates and enzymatic activation-dependent chemicals of public health concern (Hahon *et al.*, 1979; Hahon, 1984; Hahon and Booth, 1986a,b). Furthermore, recent compiled data indicate an excellent correlation between the mutacarcinogenic potential of chemicals and inhibition of α/β interferon induction (Sonnenfeld, 1986). An inchoate attempt was made, therefore, to elucidate the biologic interaction between nitrosated coal dust and a cellular defense mechanism, the interferon system.

This report describes the effect of nitrosated coal dust extract with and without microsomal enzymatic activation on viral interferon induction in mammalian cell cultures in conjunction with its mutagenic activity.

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 human Chang conjunctival (clone 1-5c-4) cell lines 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.

Rat S9. Liver homogenate 9000*g* supernatant fraction (S9) for use in interferon experiments and mutagenicity assays was prepared from the livers of male Wistar/Lewis rats (225 g/rat) after pretreatment with Aroclor 1254 (i.p. 500 mg/kg

body wt) as described by Ames *et al.* (1975). For use in experimental tests, 0.5% suspension of S9 was prepared in maintenance medium and then passed through a Nalge filter unit 0.45 μm (Nalge Co., Rochester, NY) to obtain sterile preparations. After filtration, the suspension contained 77 μg protein/ml (Lowry *et al.*, 1951).

Coal dust extracts, nitrosation, and fractionation. Coal dust used in this study was Hi Vol.A bituminous from the York Canyon seam of Colfax County, New Mexico, which was kindly provided by the Coal Research Section of the Pennsylvania State University. The coal dust extraction and nitrosation procedures closely followed those described by Whong *et al.* (1983).

Prior to fractionation of nitrosated coal dust extract, 5-ml amounts of extract were mixed with equal quantities of horse serum (GIBCO, Grand Island, NY) and agitated at 35°C for 5 days. Then, 4 ml of nitrosated extract–horse serum preparation was mixed in equal volume with either saline or 2 mM disodium ethylenediamine tetraacetic acid (EDTA) and stirred at 4°C for 24 hr to chelate metals complexed with serum proteins. Normal horse serum mixed with either saline or EDTA and handled in a similar manner served as controls. All mixtures were fractionated on Sephadex G-25M PD-10 columns (Pharmacia Fine Chemicals, Uppsala, Sweden), with a cutoff of 25,000 Da, by elution with 3 void vol of double-distilled water. To ensure sterility, all mixtures and fractions were passed through Millex GS 0.45-μm filters (Millipore Corp., Bedford, MA). Initial horse serum mixtures and the three corresponding fractions from each mixture were tested for their influence on viral interferon induction and for mutagenic activity.

Interferon induction. Duplicate experiments were performed and the procedure generally used to study the effects of coal dust test samples on interferon induction was carried out as follows: either coal dust (0.1 to 5 mg), solvent extracts, nitrosated extracts, or fractionated samples thereof, at prescribed concentrations, were added in 1-ml amounts to 9 ml volume of maintenance medium with or without 0.5% S9. These preparations were then added to 75-cm^2 plastic flasks containing confluent LLC-MK_2 cell monolayers ($\sim 2 \times 10^7$ cells) which were incubated at 35°C for 20 hr. Thereafter, 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 (MOI) was approximately 2.0. Inoculum was removed and 10 ml of maintenance medium was added to each flask. After incubation at 35°C for 20 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 phosphate-buffered saline (PBS), pH 7.1, at 4°C for 24 hr. Fluids were passed through Millex filters GV, 0.22 μm, (Millipore Corp.) 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). Controls, consisting of cell monolayers which were not treated with coal dust or extracts thereof, were handled exactly as described above.

Neutralization tests with antihuman α and β interferon antisera (Nutritional

Biochemicals, Cleveland, OH), using constant serum and varied interferon concentrations (WHO, 1984), indicated that viral-induced interferon from cell cultures was a mixture of α and β interferons. The solvent extract, dimethyl sulfoxide and nitrite solution, at concentrations used in experimental tests were neither detrimental to cell viability nor to viral induction of interferon.

Interferon assay. An immunofluorescent cell-counting assay that had been described previously (Hahon, 1981) was employed to determine the interferon potency of test samples by using 1-5c-4 cell monolayers. A 50% or greater decrease of interferon by test samples from the control, which exceeds 98% confidence limits of the assay (Hahon *et al.*, 1975), was considered significant.

Mutagenicity assay. Mutagenic activity of test samples was assessed by using the standard *Salmonella*/microsome assay with tester strain TA98 (Ames *et al.*, 1975).

RESULTS

Preliminary observations on the effect of coal dust per se on interferon induction by influenza virus in primate cell monolayers revealed that from 1 to 5 mg inhibited the induction process by more than 60% (Table 1). Quantities less than 1 mg resulted in progressively lower than 50% inhibition. The aqueous supernatant of coal dust suspensions did not contain soluble ingredients capable of depressing viral interferon induction.

Nitrosated and acidified coal dust per se and suitable control preparations thereof were also tested for their effect on viral induction of interferon. Results (Table 2) show a marked and comparable reduction of interferon induction by coal dust that was either nitrosated and acidified, acidified only, or untreated. Neither nitrosation nor the presence of an enzymatic activator (S9) in coal dust suspensions increased the inhibitory activity to interferon induction.

To test for inherent soluble components that comprise the heterogeneous nature of coal dust, extracts were prepared using polar (methanol + acetone; M + A) and nonpolar (dichloromethane; DCM) solvents. A mixture of the extracts was also nitrosated under acidic conditions and all extracts, subsequently, were as-

TABLE 1
EFFECT OF COAL DUST ON INTERFERON (IFN) INDUCTION BY INFLUENZA VIRUS IN LLC-MK_2 CELL MONOLAYERS

Coal dust (mg)	IFN[a] ($ICDD_{50}$)	IFN inhibition[b] (% ± SEM)
5	440	76.5 ± 7.0
1	700	62.6 ± 4.3
0.5	1050	43.9 ± 5.4
0.1	1670	10.7 ± 2.7
0 (Control)	1870	0 ± 0.0
Aqueous supernatant	1800	3.8 ± 2.6

[a] Reciprocal of 50% infected cell-depressing dilution of IFN/10 ml medium.
[b] Reciprocal: IFN $ICDD_{50}$/control IFN $ICCD_{50}$—1.0 × 100.

TABLE 2
EFFECT OF NITROSATED COAL DUST ON INTERFERON (IFN) INDUCTION BY INFLUENZA VIRUS IN LLC-MK_2 CELL MONOLAYERS

Treatment of coal dust (5 mg)	IFN[a] ($ICDD_{50}$)	IFN inhibition[b] (% ± SEM)
Nitrosated and acidified[c]	340	71.2 ± 8.3
Nitrosated and acidified, +S9[d]	290	75.5 ± 2.6
Acidified	380	67.8 ± 6.7
Acidified +S9	380	67.8 ± 5.5
Untreated	370	68.7 ± 8.1
Untreated +S9	350	70.4 ± 0.8
Controls:		
$NaNO_2$ (1.5 mg) in PBS[e]	1150	2.6 ± 2.6
$NaNO_2$ (1.5 mg) in PBS +S9	1110	6.0 ± 6.0
Maintenance medium	1180	0.3 ± 0.3
Maintenance medium +S9	1180	0.0 ± 0.0

[a] Mean reciprocal value of two determinations of 50% infected cell-depressing dilution of IFN/10 ml medium.

[b] Reciprocal: IFN $ICDD_{50}$/control IFN $ICDD_{50}$ (1180)—1.0 × 100.

[c] pH 3.5.

[d] Rat liver homogenate 9000*g* supernatant fraction (0.5%).

[e] Phosphate-buffered saline.

sessed for inhibition of interferon induction and mutagenicity. Results (Table 3) show that M+A and DCM solvent extracts minimally affected the interferon induction without S9, although marked inhibition occurred with nitrosated extract. In the presence of S9, all extracts were significantly ($P < 0.05$) inhibitory for interferon induction exceeding the activity of corresponding extracts without S9. Polar and nonpolar extracts, therefore, contained compounds requiring metabolic activation to inhibit interferon induction. Both activation-dependent and -independent compounds were present in nitrosated extract. When assayed for mutagenicity, both DCM and M+A solvent extracts, with or without S9, were mutagenic but weaker in activity than nitrosated extracts (Table 4). However, both polar and nonpolar extracts with S9 showed significantly ($P < 0.05$) greater activity than that noted without S9. Nitrosated extracts were comparable in mutagenicity either with or without S9.

Nitrosated coal dust extract was subjected to further extraction with horse serum which can form soluble complexes with heavy metals and polycyclic aromatic hydrocarbons. A metal chelator, EDTA, was added to serum extract to help detect metal–serum complexes. Serum extracts were fractionated on a Sephadex PD-10 column. Results (Table 5) show an overall 50% or more inhibition of viral interferon induction by both horse serum plus EDTA and horse serum plus saline extracts as well as their corresponding fractions. Apparently, the soluble chemicals that complexed with serum did not require metabolic activation as evidenced by the overall equivalency of interferon induction inhibition noted with or without the presence of S9. The comparable inhibitory activity of fraction 3, both in horse serum with or without EDTA, indicates that metal–serum complexes

TABLE 3
EFFECT OF COAL DUST EXTRACTS AND NITROSATED EXTRACTS ON INTERFERON (IFN) INDUCTION BY INFLUENZA VIRUS

Coal dust extraction	Test quantity (μg)	−S9 IFN[a] ($ICDD_{50}$)	−S9 IFN[b] inhibition (%)	+S9[c] IFN ($ICDD_{50}$)	+S9[c] IFN inhibition (%)
Dichloromethane	2.48	1310	25.2	ND[d]	
(DCM)	1.24	1760	0.0	ND	
	0.62	1800	0.0	450	73.6[e]
	0.31	1750	0.0	760	55.3
	0.15	1750	1.2	1550	8.9
Methanol + Acetone	2.20	1220	30.3	580	65.9[e]
(M + A)	1.10	1770	0.0	630	63.0
	0.55	1730	1.2	1000	41.2
	0.27	1680	4.0	1700	0.0
	0.13	1750	0.0	1720	0.0
Nitrosated	2.28	590	66.3[g]	530	68.7[e,f]
[DCM + (M + A)]	1.14	770	55.9	370	78.0
	0.57	900	48.6	440	73.8
	0.28	1750	0.0	700	58.9
	0.14	1750	4.0	1200	29.5
Controls					
Maintenance medium					
(−S9/+S9)	0.0	1750	0.0	1700	0.0
Dimethyl sulfoxide	0.5%				
+ $NaNO_2$	2.7 mg	1800	0.0		

[a] Mean reciprocal value of two determinations of 50% infected cell-depressing dilution of IFN/10 ml medium from LLC-MK_2 cell monolayers.
[b] Reciprocal: IFN $ICDD_{50}$/control IFN $ICDD_{50}$—1.0 × 100.
[c] Rat liver homogenate 9000*g* supernatant fraction (0.5%).
[d] Not determined.
[e] Paired Student's *t* test significant overall at level $P < 0.05$ (+S9 vs −S9) for each group.
[f] Significant ($P < 0.05$) from M + A group (+S9).
[g] Significant ($P < 0.05$) from DCM and M + A groups (−S9).

were either absent or, if present, were ineffectual. However, other low-molecular-weight compounds may be present in these fractions to account for the inhibitory activity to interferon induction noted. The unfractionated control (horse serum plus EDTA) and fractions thereof had no apparent effect on interferon induction.

Significant mutagenic activity was detected in horse serum plus EDTA and horse serum plus saline extracts of nitrosated coal dust when assessed without S9 (Table 6). In general, this complemented results of interferon induction inhibition noted previously. There was no significant difference in mutagenicity between serum plus EDTA and serum plus saline extracts and their corresponding fractions. That the third fraction of both serum extracts (EDTA and saline), usually associated with low-molecular weight components, showed no mutagenic activity

TABLE 4
MUTAGENIC RESPONSE OF *S. typhimurium* TA98 TO COAL DUST EXTRACTS AND NITROSATED EXTRACTS

Coat dust extraction	Test quantity (μg)	His$^+$ revertants/plate[a]	
		−S9	+S9
Dichloromethane	312.0	27	69[b]
(DCM)	156.0	22	84
	78.0	19	72
Methanol + acetone (M + A)	275.0	36	70[b]
	137.5	32	79
	68.7	26	67
Nitrosated	287.0	939[d]	1207[c,d]
[DCM + (M + A)]	143.5	732	818
	71.7	609	520
	35.8	488	224
Controls			
Dimethyl sulfoxide (0.1 ml)		13	24
2-Aminoanthracene	2.5		1359

[a] Mean of two determinations.
[b] Paired Student's t test; significant overall at level $P < 0.05$ (+S9 vs −S9) for each group.
[c] Not significant (+S9 vs −S9).
[d] Significant ($P < 0.05$) from DCM and M + A groups (±S9).

tends to support the belief that metal–serum complexes were either not formed or, if present, were not mutagenic.

DISCUSSION

Previous studies have described the potential carcinogenic content of coal dust (Falk and Jurgelski, 1979) and demonstrated the mutagenic and genotoxic activities of nitrosated coal dust extract (Whong *et al.*, 1983; Tucker *et al.*, 1984) which add support to epidemiologic findings on the possible association between coal dust and risk to gastric carcinogenesis in coal miners. Our findings confirm the mutagenicity of nitrosated and acidified coal dust extract (mixture of polar and nonpolar solvent extracts) and also reveal an affected biologic activity that pertains to a cellular defense mechanism, the interferon system. Viral induction of interferon in mammalian cell cultures was markedly inhibited by the extract, independent of enzymatic activation (S9), but inhibition was significantly increased ($P > 0.05$) in the presence of S9. This suggests biotransformation of some compounds formed by nitrosation of coal dust extract to bioactive agents resulting from their interaction with microsomal enzymes. It implies also that the interferon induction process was sensitive to adverse activation-independent chemicals, e.g., nitrosamides, oxides of PNAs, and activation-dependent compounds, e.g., nitrosamines and PNAs, that were derived from coal dust by solvent extraction and potentially formed by nitrosation thereof.

Our findings were comparable to those of Whong *et al.* (1983) who reported

TABLE 5
FRACTIONATED HORSE SERUM EXTRACTS (HSE) OF NITROSATED COAL DUST: EFFECT ON INTERFERON (IFN) INDUCTION BY INFLUENZA VIRUS

HSE[a]	Test dilution	−S9 IFN[b] ($ICDD_{50}$)	−S9 IFN[c] inhibition (%)	+S9[d] IFN ($ICDD_{50}$)	+S9[d] IFN inhibition (%)
HSE + EDTA[e]					
Unfractionated	1:20	460	59.3	400	80.0[f]
Fraction 1	1:10	220	80.6	500	75.0
Fraction 2	Undil	280	75.3	520	74.0
Fraction 3	Undil	380	66.4	780	61.0
HSE + Saline					
Unfractionated	1:20	420	62.9	520	75.0[f]
Fraction 1	1:10	390	65.5	620	69.0
Fraction 2	Undil	300	73.5	700	65.0
Fraction 3	Undil	500	55.8	1000	50.0
Control HS + EDTA					
Unfractionated	Undil	1040	8.0	2300	0.0[f]
Fraction 1	Undil	1000	12.6	2050	0.0
Fraction 2	Undil	1100	2.7	2000	0.0
Fraction 3	Undil	1130	0.0	1950	2.5
Control–maintenance medium (−S9/+S9)		1130	0.0	2000	0.0

[a] Nitrosated and acidified mixture of dichloromethane and methanol + acetone extracts of coal dust.

[b] Reciprocal of 50% infected cell-depressing dilution of IFN/10 ml medium from LLC-MK_2 cell monolayers.

[c] Reciprocal: IFN $ICDD_{50}$/control IFN $ICDD_{50}$—1.0 × 100.

[d] Rat liver homogenate 9000*g* supernatant fraction (0.5%).

[e] Disodium ethylenediaminetetraacetic acid.

[f] Paired Student's *t* test; not significant overall at level $P < 0.05$ for each group (+S9 vs −S9) or for HSE (EDTA vs saline) ± S9.

that nitrosated coal dust extract exhibited mutagenic activity that was activation-independent with no obvious enhancement of mutagenicity by S9. It can be inferred, therefore, that only direct-acting mutagens were present in the extract. However, the possibility exists that the apparent inability to detect metabolic activation-dependent mutagenic compounds in nitrosated coal dust extract, at variance with interferon induction results, may be a reflection of the bacterial tester strain used or the need for subtle test modifications, e.g., incubation conditions, S9 source, mutagenicity-enhancing factors (ATP, riboflavin). A comparison of the quantities of coal dust extract used to assess for mutagenic activity and for inhibition of interferon (Tables 3 and 4) revealed that the former required milligram amounts to obtain a detectable response while the latter was achieved with microgram quantities. It would appear that the interferon induction process in mammalian cells was highly sensitive to the adverse effects of chemical com-

TABLE 6
MUTAGENIC RESPONSE OF *S. typhimurium* TA98 TO FRACTIONATED HORSE SERUM EXTRACTS (HSE) OF NITROSATED COAL DUST

HSE[a]	Test quantity/ml			
	0.1	0.05	0.025	0.0125
HSE + EDTA[b]				
Unfractionated	1553[c]	1271	909	611[d]
Fraction 1	1050	663	422	217
Fraction 2	252	148	98	69
Fraction 3	25	42	21	24
HSE + Saline				
Unfractionated	1461	1184	776	489[d]
Fraction 1	922	740	370	239
Fraction 2	250	148	88	54
Fraction 3	24	22	25	28
Control HS + EDTA				
Unfractionated	29	27	22	23
Fraction 1	19	23	25	26
Fraction 2	25	17	17	25
Fraction 3	25	24	26	18
Controls				
Dimethyl sulfoxide (0.1 ml)	20			
2-Aminoanthracene (2.5 μg)	3400			

[a] Nitrosated mixture of dichloromethane and methanol + acetone extracts of coal dust.
[b] Disodium ethylenediaminetetraacetic acid.
[c] His^+ revertants/plate. Mean of two determinations.
[d] Paired Student's *t* test; not significant overall at level ($P < 0.05$) between groups.

pounds formed by nitrosation of coal dust extract whether they were mutagenic or of questionable mutagenicity.

Nitrosated and acidified coal dust extract was further subjected to extraction with horse serum and serum containing a metal chelator, EDTA. Organic compounds and soluble complexes of carcinogenic metals are known to bind to proteins (Heath *et al.*, 1969; Heidelburger, 1975). When serum extracts are fractionated on Sephadex columns, the first fraction contains the majority of serum protein (higher molecular weight components), the second contains the remaining protein and some low-molecular-weight compounds, and the third fraction contains only low-molecular-weight components (Chrisp *et al.*, 1978). All fractionated products of serum, with or without EDTA, markedly and comparably inhibited viral interferon induction. The overall equivalency in interferon induction inhibition noted, either with or without S9, indicates that the soluble chemicals which complexed with serum were activation-independent. Furthermore, the comparable results with low-molecular-weight-binding components to serum with or without EDTA suggests that, with an extract of this particular bituminous coal dust, metal–serum complexes were either not present or were ineffectual for increasing the inhibition of interferon induction. In general, the mutagenic activity noted with identical serum fractions conformed to the above findings.

Coal dust per se, while inhibiting viral interferon induction, did not show increased inhibitory activity whether it was nitrosated, acidified, or both, or exposed to enzymatic activation. Although particulates of public health concern have been reported to affect adversely interferon induction (Hahon and Eckert, 1976; Hahon *et al.*, 1983), the limitations of the *Salmonella*/microsome assay with respect to particulate matter precluded an assessment of mutagenicity for coal dust per se. It is assumed that the hydrocarbon components of coal dust associated with potential deleterious biologic effects may be inherently bound and inaccessible to nitrosation. To account for this circumstance in terms of the association between swallowed coal dust and gastric carcinogenesis, minute amounts of promutagens and procarcinogens may be present on particulate surfaces for nitrosation. For adverse health effects to occur, cumulation of active mutacarcinogens dependent on continuous and long-term exposure may be a requisite.

It has been suggested that interferon is produced continuously in the healthy host by alimentary tract mucosal lymphoid tissue in response to contact with lectins, heterologous proteins, and other xenobiotics which may function as interferon inducers (Bocci, 1981). Experimental studies have demonstrated antiviral activity, characteristic of interferon, in abdominal lymph of healthy rabbits (Bocci *et al.*, 1984). Presently, the physiological role of interferon in abdominal tissue is unknown. Interferon's antiviral, anticellular, and immunomodulatory functions have been well documented (Taylor-Papadimitriou, 1980), and it could, conceivably, influence or help maintain the normal physiologic defenses of the alimentary tract, i.e., immune barrier/response and local antibodies. Whether impairment of interferon production by a xenobiotic agent, i.e., nitrosated coal dust, is relevant to gastric carcinogenesis has yet to be elucidated.

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