CHAPTER 7

MECHANISMS OF ASBESTOS AND NONASBESTIFORM PARTICLES AND FIBERS IN BRONCHOGENIC CARCINOMA

Brooke T. Mossman, PhD and John E. Craighead, MD

Department of Pathology
University of Vermont
Burlington, Vermont

INTRODUCTION

Bronchogenic carcinoma is the leading cause of fatal cancer and the most common malignancy of the respiratory tract in cigarette smokers. In comparison to smokers in the general population (8- to 10-fold greater than normal risk), the risk of bronchogenic carcinoma is substantial (80- to 92-fold) in asbestos workers who smoke [1,2]. In contrast, the incidence of tumors in the nonsmoking asbestos worker is less (1.5 to 4-fold) [1–4].

The role of other minerals as cofactors in bronchogenic carcinoma has been implicated by a number of experimental studies [5,6]. Polycyclic aromatic hydrocarbons (PAH) (i.e., chemical carcinogens in cigarette smoke), when adsorbed to a variety of particles such as hematite, aluminum oxide and carbon and placed via intratracheal instillation into rodents, are more carcinogenic than identical amounts of PAH administered alone. Presumably, the dusts facilitate the penetration of the chemicals and their persistence in the respiratory tract [7]. Because instillation of particles does not cause tumors,

"carrier" dusts are referred to as (co)carcinogens*, as opposed to complete carcinogens such as PAH.

The experimental approaches described here were developed to determine mechanisms of mineral-induced (co)carcinogenicity at the cellular level. We were interested specifically in elucidating the interaction of selected fibers and particles with "target" epithelial cells of the airways in vitro. In addition, we developed a tracheal graft model to demonstrate the tumorigenic potential of airway cells exposed to minerals alone and in combination with PAH. Our emphasis thus far has been on the characterization of asbestos-induced cellular responses, although a number of other minerals (i.e., fiberglass, hematite, kaolin) have been examined in comparative studies.

APPROACHES

A serious shortcoming of whole animal models after intratracheal instillation or inhalation of asbestos is the small numbers and different histological types of tumors appearing despite protracted periods of exposure [8]. To address this problem, we developed methods for long-term organ culture of rodent tracheal epithelium after exposure in vitro to weighed amounts (mg) of minerals (Figure 1). An inbred hamster (15.16_{EHS}, Research Triangle, NC) was selected as the species of interest to allow implantation of tissues subcutaneously into donor animals. Moreover, after intratracheal instillation of PAH, hamsters develop bronchogenic neoplasms, the type of tumor observed most often in the respiratory tract of man.

After removal of the trachea from animals, tracheal organ cultures are prepared and exposed to minerals alone or coated with PAH. They then are maintained in culture for up to 6 months. Tissues are removed at intervals for morphologic assessment using histology, autoradiography and transmission and scanning electron microscopy (TEM and SEM) [9–11]. Other trachea are grafted subcutaneously into weanling hamsters that are palpated for tumors on a regular basis. When neoplasms appear, grafts are removed for histology and step-sectioned to determine the type of tumor and its site of origin from the tracheal epithelium [12–14].

To enable quantitative biochemical studies, cloned lines of tracheal epithelial cells have been established from neonatal hamsters [15,16]. Thus, we are able to look at enzymatic changes occurring after addition of minerals [17,18] and their effects on metabolism and uptake of PAH [8,19,20].

---

*(Co)carcinogenesis is defined as the ability of a mineral to cause tumors in combination with a PAH.

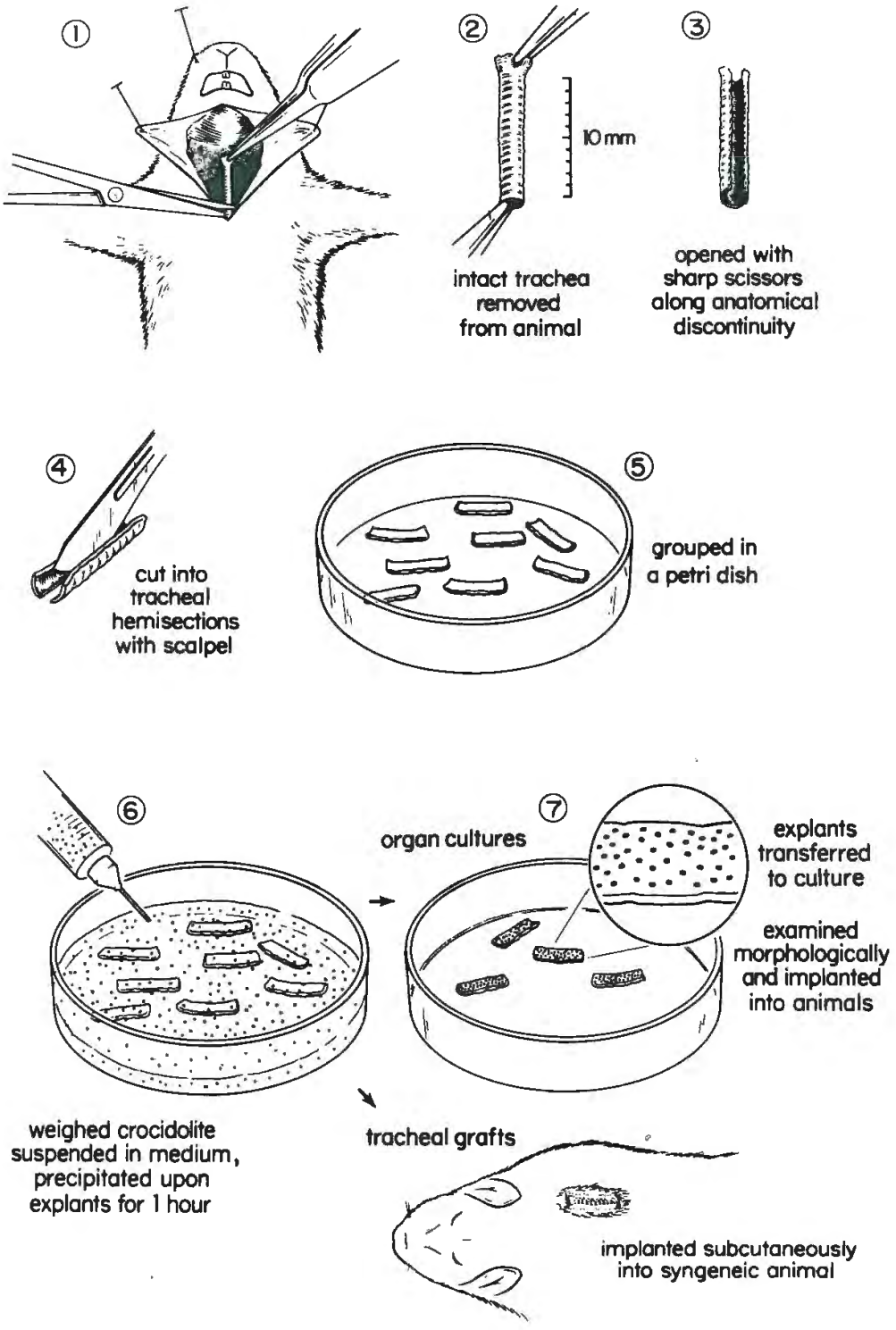

Figure 1. Schematic diagram of the procedure for preparation of hamster tracheal organ cultures. After exposure for 1 hour to minerals alone and coated with PAH, the explants are maintained in vitro for periods of as long as 6 months. Other tissues are implanted into syngeneic animals to determine their tumorigenic potential.

RESULTS AND DISCUSSION

Comparative Studies to Determine the (Co)carcinogenicity of Crocidolite Asbestos, Carbon, Kaolin and Hematite in Implanted Tracheal Organ Cultures

The carcinogenicity of the PAH 3-methylcholanthrene (3MC) was examined in hamster tracheal epithelium after its adsorption at equal amounts onto crocidolite asbestos (UICC reference sample), carbon (0.5–1.0 μ diameter, IIT Research Institute, Chicago, IL), kaolin (3–5 μ diameter, Georgia Kaolin Co., Elizabeth, NJ) and hematite (0.5–1 μ diameter, IIT Research Institute, Chicago, IL) [13]. Dusts alone (16 mg/ml) and coated with 3MC were suspended in Hanks' Balanced Salt Solution (HBSS) at 2 concentrations (ca 8 and 16 mg/ml) before precipitation on the tracheal epithelium for 1 hour. Use of radioactively tagged 3MC made it possible to determine the amounts adsorbed to each type of particulate and the dosage to the tissues. After maintenance for 4 weeks in vitro, explants were grafted into syngeneic animals.

Neoplasms, the majority of which were carcinomas, appeared after exposure of tissues to 3MC-coated materials, but not after exposure to particulates alone (Figure 2). At highest concentrations (16 mg/ml), the relative (co)carcinogenicity of the dusts was crocidolite $>$ hematite $>$ kaolin $>$ carbon. However, at lowest amounts (8 mg/ml) no statistical differences between groups were observed.

We hypothesized that (co)carcinogenicity might be related to either the affinity of the dust for 3MC or its elution into culture medium. However, the relative amounts of 3MC adsorbed to and released from various particulates was not related consistently to tumorigenic potential. For example, carbon adsorbed more hydrocarbon than identical amounts of other dusts, although elution was minimal. In contrast, crocidolite neither adsorbed nor released large amounts of 3MC. These results were intriguing and prompted experiments to determine possible mechanisms of mineral-induced (co)carcinogenicity at the cellular level.

Concepts of Carcinogenesis and Promotion

Carcinogenesis is thought to be a progression of events consisting of sequential stages of initiation and promotion. These processes were demonstrated originally in the classical mouse skin system but are believed to apply to a variety of organs including liver and respiratory tract [21]. An "initiator" is defined as an agent capable of interacting with DNA. This molecular instability results in a cell that is prepared for the sequential

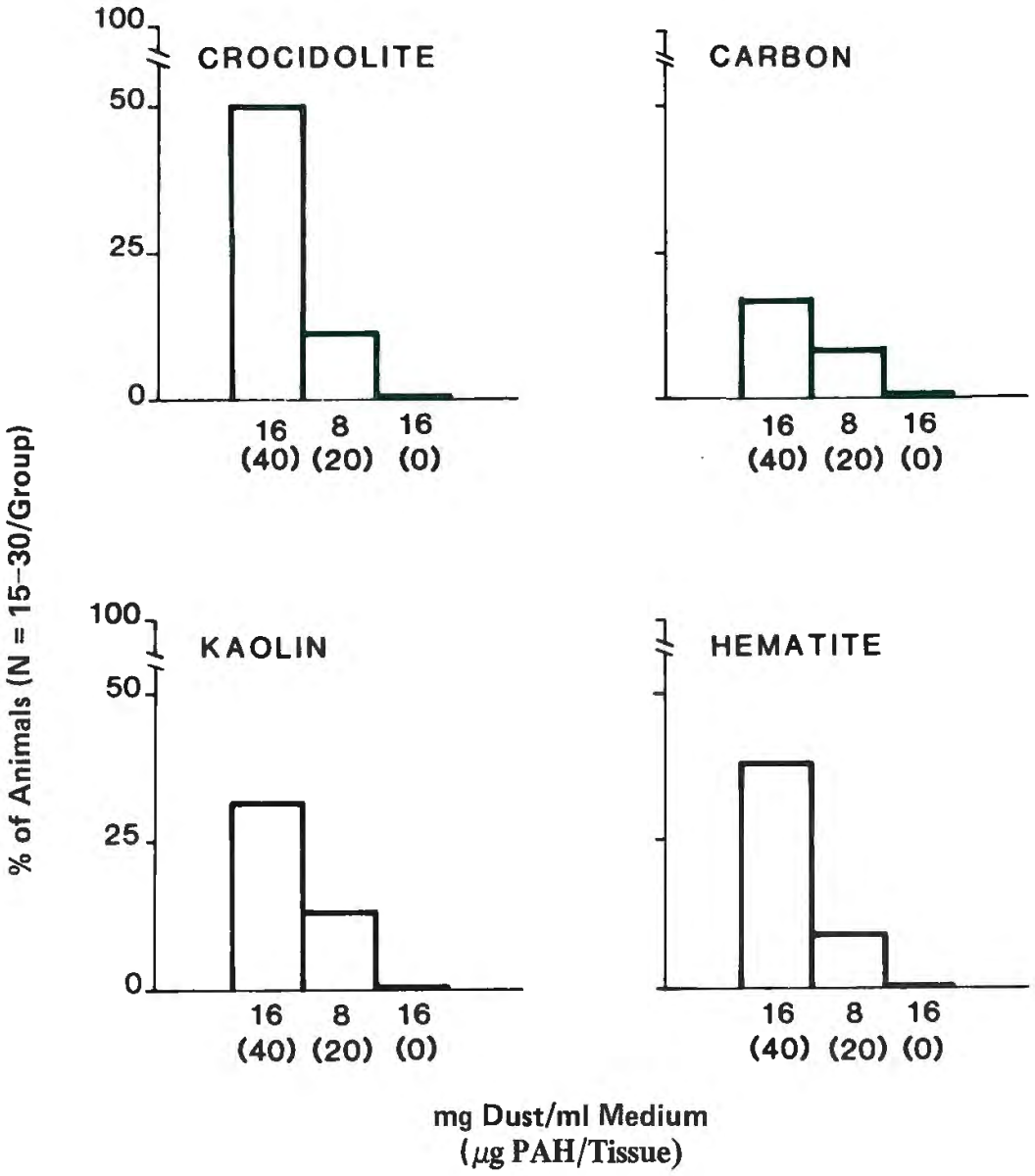

Figure 2. Tumors occurring from implanted hamster trachea after exposure in vitro to 3-methylcholanthrene (3MC) coated on crocidolite asbestos, carbon, kaolin and hematite.

progression to malignancy. In contrast, "promoters" are defined as a subset of (co)carcinogens that lack significant carcinogenic activity but result in the enhancement of tumors when applied after a subcarcinogenic dose of an initiator. Promoters, such as the phorbol esters in mouse skin, cause proliferative and biochemical cellular alterations that are necessary for the development of tumors (Table I). These properties include: (a) attachment

Table I. Properties of Classical Tumor Promoters Important in Carcinogenesis

1. Interaction with an entrance into the target cell
2. Stimulation of cellular division
3. Inhibition of normal cell differentiation
4. Increased induction of ornithine decarboxylase (ODC), the rate-limiting enzyme in the biosynthesis of polyamines

to and entrance into target cells; (b) stimulation of cellular division, a necessary event for the transformation of cells by chemical carcinogens [22]; (c) inhibition of normal cell differentiation; and (d) induction of ornithine decarboxylase (ODC), the rate-limiting enzyme in the biosynthesis of polyamines. A critical intracellular level of polyamines is necessary for normal cell division and maturation.

Our investigations thus far have focused on the biochemical and morphologic effects of both crocidolite and chrysotile asbestos on tracheal epithelial cells in vitro. Both types of asbestos and a variety of nonasbestos dusts [9–11] are phagocytized by superficial tracheal epithelial cells and transported to underlying basal cells, the presumed progenitors of bronchogenic carcinoma (Figure 3). Moreover, both crocidolite and chrysotile cause proliferative alterations, including enhancement of serum-dependent cell division and an increase in ODC activity in monolayers of tracheal epithelial cells [17]. In studies now in progress, we are examining comparatively the effects on induction of ODC by nontoxic amounts of crocidolite, chrysotile, kaolin and hematite (Table II). Induction of ODC is comparable after addition of kaolin, chrysotile or crocidolite to cultures, whereas hematite does not stimulate the enzyme.

Table II. Effects of Minerals on Induction of ODC When Added to Tracheal Epithelial Cells for 24 Hours

Mineral	Concentration ($\mu g/cm^2$)[a]	ODC Activity (nm CO_2/hr/mg protein)
Crocidolite	2.56	0.021±0.002
Chrysotile	0.65	0.025±0.002
Kaolin	2.56	0.029±0.009
Hematite	2.56	0.016±0.010
Control	–0–	0.011±0.004

[a] μg of material per cm^2 of surface area in Petri dish.

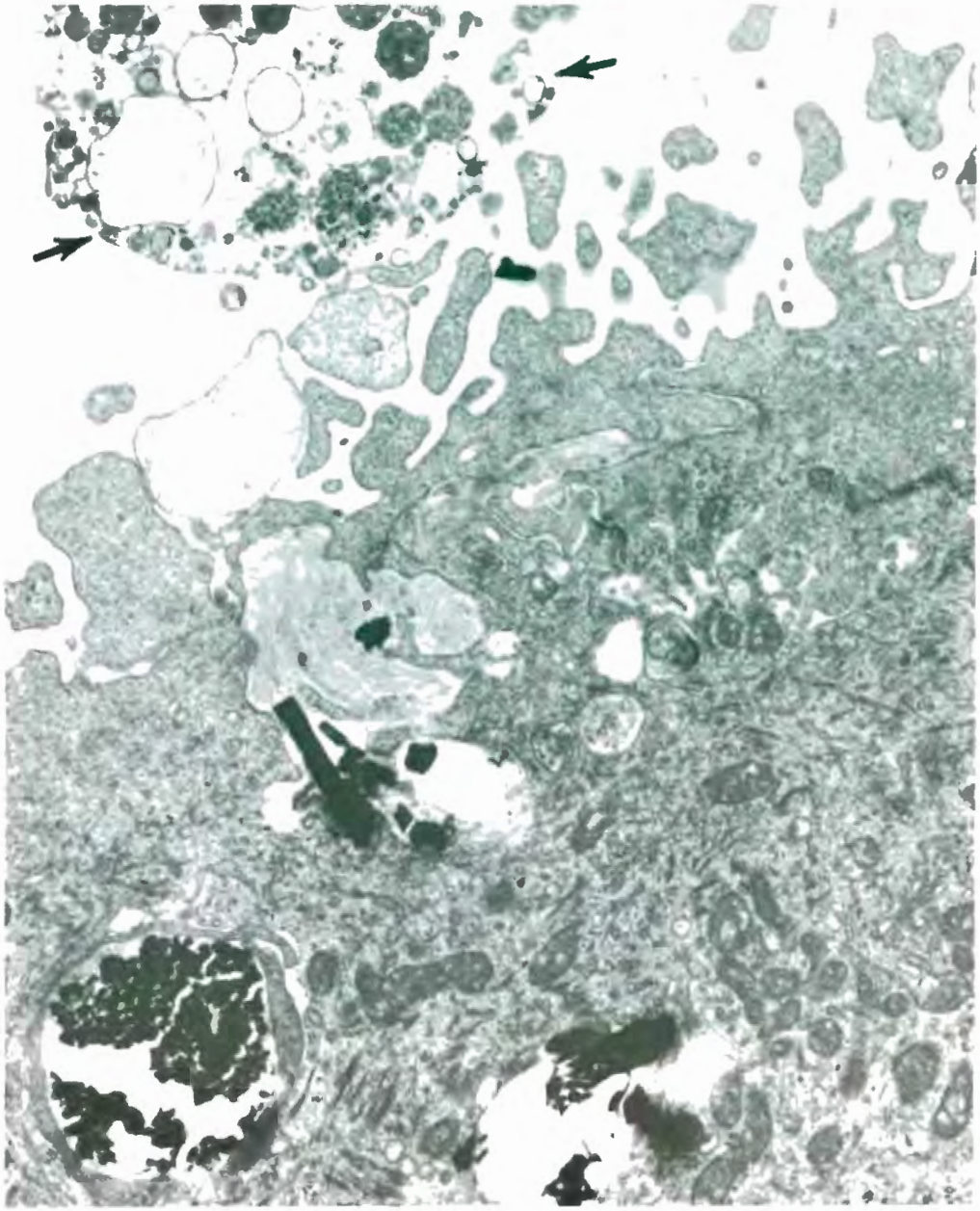

Figure 3. A transmission electron micrograph showing intracellular accumulations of crocidolite asbestos in a basal cell. Note the degenerating superficial cell (arrows). Uranyl acetate and lead citrate. X12,000.

After precipitation on tracheal organ cultures, crocidolite causes increased incorporation of ^{3}H-thymidine, basal cell hyperplasia and the appearance of squamous metaplasia, the conversion of a normal mucociliary epithelium to a squamous, cornified layer (i.e., inhibition of normal cell differentiation) (Figure 4) [23]. These proliferative alterations are observed also with the use

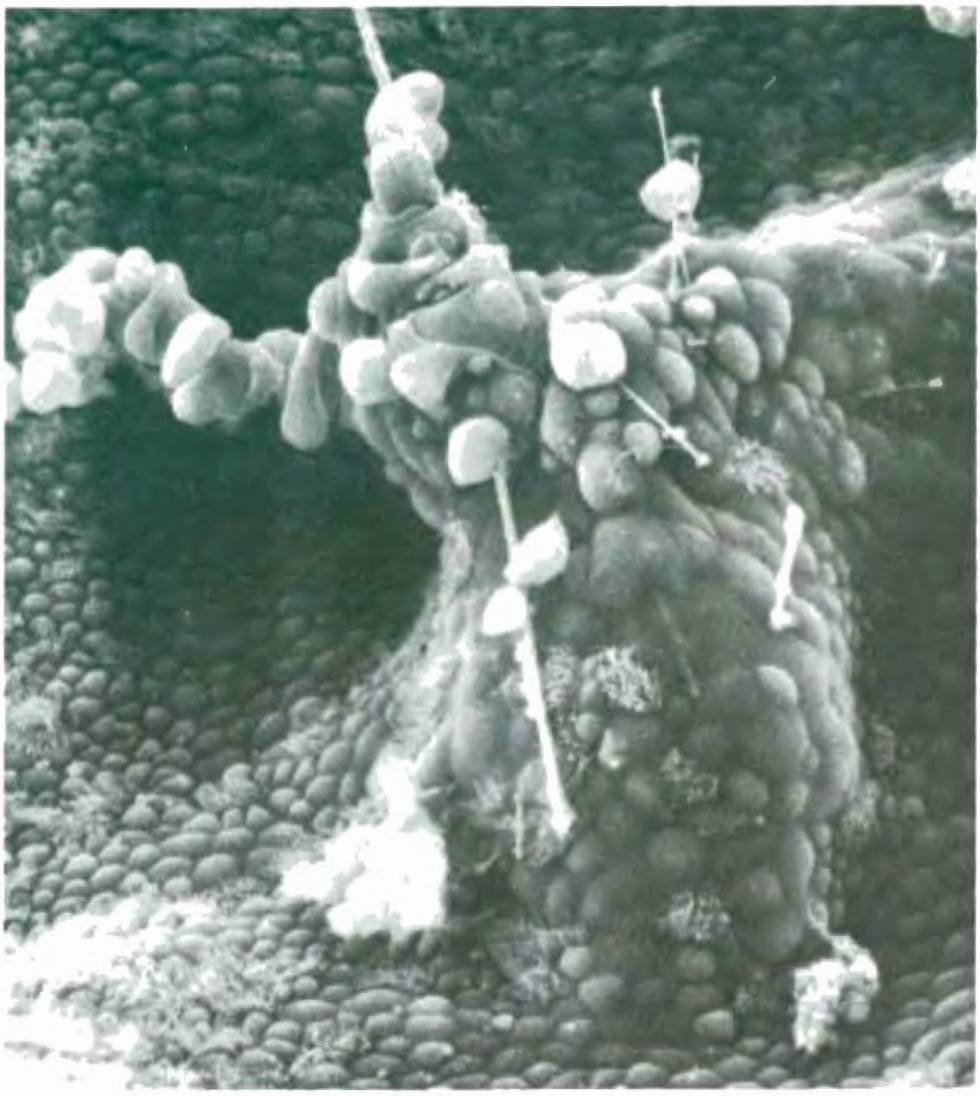

Figure 4. A scanning electron micrograph showing proliferation of tracheal epithelial cells in organ culture around fibers of crocidolite asbestos. The explant was exposed to crocidolite in 4.0 mg/ml medium and examined after 4 weeks in culture. ×800.

of other needle-like fibers, including borosilicate glass [12] and amosite asbestos [23]. In contrast, metaplastic changes do not occur using chrysotile, a pliable curly fiber, and nonasbestos particles such as hematite, kaolin and carbon.

Although crocidolite exhibits many of the characteristics of classical tumor promoters on target cells, additional mechanisms must be considered in the context of mineral-induced (co)carcinogenesis (Table III). For example, exposure to cytotoxic dusts might result in damage to cells of the airways and impairment of normal mucociliary clearance. Thus, both particulates and

Table III. Properties of Minerals that Might Be Important in Carcinogenesis

1. Damage to cells of the respiratory tract resulting in impairment of clearance mechanisms and increased retention of minerals
2. Ability to adsorb and release PAH
3. Efficiency of transfer of PAH to cells
4. Effects on metabolism of PAH

chemical carcinogens would be retained for extended time in the respiratory tract.

The interactions between PAH and minerals also can be important. PAH must be metabolized to active forms, which bind to DNA, and water-soluble forms, which are excreted from the cell. Our recent studies [8] suggest that asbestos may play a role in the metabolism of PAH by tracheal epithelial cells. In these experiments, cells in culture were monitored for the activity of aryl hydrocarbon hydroxylase (AHH), a system of microsomal P-450 enzymes responsible for metabolism of PAH. After addition of the PAH 3MC, a greater than two-fold increase of normal AHH activity was observed. However, 3MC-induced activity was enhanced significantly when crocidolite fibers and 3MC were added simultaneously to cultures.

In current studies, we are examining uptake and retention of the PAH, benzo(a)pyrene (BaP), by tracheal epithelial cells after addition of BaP-coated minerals to cultures [20]. When BaP is adsorbed to chrysotile or crocidolite before their addition to cultures, 70% of the total BaP introduced enters the cells within 1 hour, whereas 50% remains intracellular after 8 hours. In contrast, if identical amounts of BaP are added directly to medium, an initial influx of 20% is observed and cells retain only 5% of the initial amount at 8 hours. Although kaolin and hematite also facilitate the initial cellular incorporation of BaP (in comparison to results obtained with solubilization of BaP in medium), their effects on the retention of the hydrocarbon are not as striking as those with use of asbestos. In contrast, after interaction of BaP-coated fiberglass (Code 100 microfiber, Johns Manville, Denver, Colorado), little cellular transfer of BaP is observed over an 8-hour period (Eastman, Mossman and Bresnick, manuscript in preparation).

SUMMARY AND CONCLUSIONS

Asbestos appears to be noncarcinogenic or weakly carcinogenic in airway epithelium unless combined with complete carcinogens such as PAH in

cigarette smoke. This conclusion is supported by both epidemiological data [1–4] and studies using rodents after intratracheal instillation of asbestos, PAH or the two in combination [24–26]. Under these circumstances, administration of asbestos alone does not cause tumors. Although neoplasms are induced with PAH, numbers are increased when equal amounts of PAH are coated on fibers before instillation. A variety of minerals can be (co)carcinogenic when coated with PAH, but to varying degrees.

The mechanisms of mineral-induced (co)carcinogenesis are complex, but can be divided into two different spheres. First is the association of the mineral with hydrocarbons and its effects on cellular transfer, metabolism and retention of the chemical carcinogens. Second are the morphologic and biochemical changes in tracheal epithelium induced by minerals such as crocidolite asbestos. This spectrum of effects appears similar to the influences of a number of classical tumor promoters on target cells.

In comparison to fiberglass and nonasbestiform materials such as kaolin, carbon and hematite, both crocidolite and chrysotile possess a variety of properties that might be essential in (co)carcinogenesis (Table IV). These include induction of proliferative alterations and enhanced transfer of PAH to epithelial cells. Because the activation of PAH and their retention by cells are increased after exposure to asbestos, the chances of an interaction between PAH and DNA—the presumed target molecule for transformation—are greater.

Table IV. Effects of Minerals that Might Be Critical in Transformation of Tracheal Epithelial Cells by PAH

	Crocidolite	Chrysotile	Fiberglass	Kaolin	Hematite
Uptake and entrance into basal cells	+[a]	+	+	+	++
Stimulation of cellular division	+	+ (transient)	E.P.[b]	–[c]	––
Inhibition of differentiation (squamous metaplasia)	+	–	+	–	––
Induction of ODC (>synthesis of polyamines)	+	+	E.P.	+	––
Transfer into and retention of PAH by cells	++	++	–	+–	+–

[a] + = mineral exhibits the property.
[b] E.P. = experiments in progress.
[c] – = mineral does not exhibit the property.

ACKNOWLEDGMENTS

Supported by Grant 00888 from the National Institute for Occupational Safety and Health. The excellent assistance of Nancy Green, Lucy Jean, Joanne Landesman and Judith Lessler is appreciated.

REFERENCES

1. Selikoff IJ, Hammond EC, Churg J: Asbestos exposure, smoking and neoplasia. JAMA 204:106–110, 1968.
2. Selikoff IJ, Seidman H, Hammond EC: Mortality effects of cigarette smoking among amosite asbestos factory workers. J Natl Cancer Inst 65:507–513, 1980.
3. Meurman LO, Kiviluoto R, Hakama M: Combined effect of asbestos exposure and tobacco smoking on Finnish anthophyllite miners and millers. Ann NY Acad Sci 330:491–496, 1979.
4. Saracci R: Asbestos and lung cancer: An analysis of the epidemiological evidence on the asbestos-smoking interaction. Int J Cancer 20:323–331, 1977.
5. Nettesheim P: Respiratory carcinogenesis studies with the Syrian golden hamster: a review. Prog Exp Tumor Res 16:185–200, 1972.
6. Nettesheim P, Topping DC, Jamasbi R: Host and environmental factors enhancing carcinogenesis in the respiratory tract. Ann Rev Pharmacol Toxicol 21:133–163, 1981.
7. Henry MC, Kaufman DG: Clearance of benzo(a)pyrene from hamster lungs after administration on coated particles. J Natl Cancer Inst 51:1961–1970, 1973.
8. Mossman BT, Craighead JE: Mechanisms of asbestos carcinogenesis. Environ Res 25:269–280, 1981.
9. Mossman BT, Adler KB, Craighead JE: Interaction of carbon particles with tracheal epithelium in organ culture. Environ Res 16:110–122, 1978.
10. Mossman BT, Ley B, Kessler JB, Craighead JE: Interaction of crocidolite asbestos with hamster respiratory mucosa in organ culture. Lab Invest 36:131–139, 1977.
11. Mossman BT, Craighead JE: Topical application of polycyclic hydrocarbons to differentiated respiratory epithelium in long-term organ cultures. In: Karbe E, Park JF, eds. Experimental lung cancer, carcinogenesis and bioassays. Berlin: Springer-Verlag, 1974, pp. 514–520.
12. Mossman BT, Craighead JE: Use of hamster tracheal organ cultures for assessing the cocarcinogenic effects of inorganic particulates on the respiratory epithelium. Prog Exp Tumor Res 24:37–47, 1978.
13. Mossman BT, Craighead JE: Comparative cocarcinogenic effects of crocidolite asbestos, hematite, kaolin and carbon in implanted tracheal organ cultures. In: Critchlow A, ed. Inhaled particles, Vol. V, London: Pergamon Press, 1982.

14. Craighead JE, Mossman BT: Carcinoma induction by 3-methylcholanthrene in hamster tracheal tissue implanted in syngeneic animals. Prog Exp Tumor Res 24:48–60, 1979.
15. Mossman BT, et al.: Isolation and spontaneous transformation of cloned lines of hamster tracheal epithelial cells. Cancer Res 40:4403–4404, 1980.
16. Last JA, Kaizu T, Mossman BT: Glycoprotein synthesis by an established cell line from hamster tracheal epithelium. Exptl Lung Res 1:89–98, 1980.
17. Mossman BT, Landesman JM, Craighead JE: Asbestos stimulates ornithine decarboxylase (ODC) activity in hamster tracheal epithelial cells. J Cell Biol 87:307a, 1980.
18. Mossman BT, Halleron PA, Craighead JE: Stimulation of Na^+-K^+ ATPase activity in tracheal epithelial cells after exposure to crocidolite asbestos. J Cell Biol 83:288a, 1980.
19. Eastman A, Mossman BT, Bresnick E: Formation and removal of benzo(a)pyrene adducts of DNA in hamster tracheal epithelial cells. Cancer Res 41:2605–2610, 1981.
20. Mossman BT, Eastman A, Landesman JM, Bresnick E: Effects of crocidolite and chrysotile asbestos on cellular uptake, metabolism and DNA after exposure of hamster tracheal epithelial cells to benzo(a)pyrene. In: Proc 2nd international conference on in vitro effects of mineral dusts, in press.
21. Marx JL: Tumor promoters: Carcinogenesis gets more complicated. Science 201:515–517, 1978.
22. Cayama E, Tsuda E, Sarma DSR, Farber E: Initiation of chemical carcinogenesis requires cell proliferation. Nature 275:60–62, 1978.
23. Mossman BT, Craighead JE, MacPherson BV: Asbestos-induced epithelial changes in organ cultures of hamster trachea; inhibition by retinyl methyl ether. Science 207:311–313, 1980.
24. Miller L, Smith WE, Berliner SW: Tests for effects of asbestos on benzo(a)pyrene carcinogenesis in the respiratory tract. Ann NY Acad Science 132:489–495, 1965.
25. Shabad LM, Pylev LN, Krivosheeva LV, Kulogina TF, Nemenko BA: Experimental studies on asbestos carcinogenicity. J Natl Cancer Inst 52:1175–1187, 1974.
26. Smith WE, Miller L, Churg J: Respiratory tract tumors in hamsters after intratracheal instillation of benzo(a)pyrene with and without asbestos. Proc Am Assoc Cancer Res 9:65, 1968.

HEALTH ISSUES RELATED TO METAL AND NONMETALLIC MINING

Edited by
WILLIAM L. WAGNER
WILLIAM N. ROM
JAMES A. MERCHANT

BUTTERWORTH PUBLISHERS
Boston • London
Sydney • Wellington • Durban • Toronto

An Ann Arbor Science Book

Ann Arbor Science is an imprint of Butterworth Publishers

Library of Congress Catalog Card Number 82-48649
ISBN 0-250-40610-1

10 9 8 7 6 5 4 3 2 1

Butterworth Publishers
10 Tower Office Park
Woburn, MA 01801

Printed in the United States of America