K-28126-(00)

Relationship between Exposure to Asbestos, Collagen Formation, Ferruginous Bodies, and Carcinoma

EARL S. FLOWERS, Ph.D.

School of Public Health, University of California, Los Angeles, Los Angeles, California 90024

Exposure to asbestos is associated with chronic fibrosing processes, formation of ferruginous bodies, and an increased risk of carcinoma. It is possible to relate the various biological responses to asbestos exposure in terms of fundamental effects on the redox state of cells or tissues. Collagen synthesis is mediated by the level of α-ketoglutaric acid, and the rate of collagen formation is directly related to the rates of fibrosis, including elastin formation. The increase in oxidative reactions associated with collagen formation is thought to increase the amount of ferric iron bound to cellular protein, resulting in formation of a silicate-iron(III) bond on an asbestos fiber. The iron-containing protein bound to the asbestos fiber by the strong silicate ionic bond is a ferruginous body. The corresponding decrease in the ferrous-ferric redox couple inhibits electron transport, thus stabilizing exoperoxides and free radicals thought to be involved in the initiation of carcinomas.

THE SEVERAL BIOLOGICAL AND TISSUE RESPONSES on exposure to asbestos have been regarded as being unrelated one to another or, specifically, as indication that the formation of ferruginous bodies was not related to chronic fibrosing processes nor to an increased risk of carcinoma. To the contrary, when the various responses to asbestos exposure are viewed in terms of molecular mechanisms, it becomes increasingly apparent that all biological responses to asbestos are intimately related. The relationship results primarily from modification in the energetic pathways of the macrophage cells that first encounter the fiber and is ultimately expressed by a perturbation and removal of ferric-containing proteins. To reestablish redox equilibrium, the macrophage cells increase oxygen consumption, and the associated increase in oxidative metabolism leads to export of a fibrogenic factor with concomitant increase in collagen, elastin, and reticulin in surrounding cells and tissues.

Development of carcinoma is poorly understood at cellular and tissue levels, but it is possible to note that disturbances in cofactor requirements for oxidoreductases and perturbation of tissue energetics most likely increase the probability for expression of a carcinogen. There is also evidence that calcium metabolism involved in energy transport by mitochondria as well as in other biochemical pathways is disrupted, leading to calcification of pleural membranes possibly due to disturbances at the hormonal level of action. With the premise that all biological responses to asbestos exposure are related, it is of interest to examine various implications of the premise from a molecular biology viewpoint.

Bioenergetics

It is possible to view defense mechanisms of cells and tissues in terms of the generation and flow of energy-rich compounds. Since biological systems derive energy by oxidation of organic substrates,[1] a fundamental response to challenge is an increase in energy production leading to increased consumption of oxygen. The observation that bronchial epithelial slices increase oxy-

...consumption in the presence of asbestos, ...ystalline silica, and polygonal glass sup...rts this general observation.[2] Ultimately, ...e consumption of oxygen leads to oxida...on of ferrous to ferric ions, and for the ...ocess to continue it becomes necessary to ...crease the level of ferric ion in order to ...eturn to a redox equilibrium as described ...y Krebs and Veech.[1] Ferric ion can be ...educed to ferrous ion which would create ...other oxidized substrates, or ferric ion could ...e removed by precipitation or complexing ...n some manner. Ferric ions are unstable ...nder alkaline conditions, and their removal ...rom solution and or their reduction are ...hermodynamically favored.[1]

It is possible that an increase in energy production associated with an increase in oxygen consumption has survival value. With an increase in energy, the tissues are perhaps better prepared for defense, the temperature may be increased, leading to increased rates of enzyme activity, and cells can export energetic compounds forming a chemotaxic energy gradient to attract macrophages, lymphocytes, and other wandering cells to aid in the defense. Aggregation of macrophages around an asbestos fiber is probably related to energetic phenomena. Hence the mobility and apparently random movement of phagocytic cells in general ...ould be associated with chemotaxic energy ...actors, representing the principle means of ...ellular communication. Bioenergetics of ...amars in relation to induction of mesothe...ioma is particularly significant and will be ...iscussed later.

Ferruginous Body Formation

A ferruginous body refers to an iron-containing protein body with a fibrous core. The bodies are thought to be formed by macrophage cells attempting to phagocytize a foreign fiber. There are various concepts regarding formation of ferruginous bodies, and the term "ferruginous" was coined by Gross in 1966.[3] This general term has resulted in part from uncertainty regarding the nature of the fiber core and observations that asbestos fibers are not unique in inducing ferruginous bodies. Ferruginous body formation has been induced by asbestos, fibrous glass, various vegetable fibers, and other silicate-containing minerals.

Ferruginous bodies occur in a variety of shapes and sizes. There can be evenly distributed deposits, a series of clumplike deposits, or large deposits on the ends assuming a barbell shape. Sizes vary, but the fiber core approximates the lengths and diameters of asbestos and other fibers found in human lungs. Formation of the ferruginous body is thought to occur by deposition of ferritin and/or hemosiderin in the ferric oxidation state on an electronegative surface.

On phagocytosis of foreign substances, macrophage cells encapsulate the substance in a mucopolysaccharide coat.[5-7] The coat appears to have a high affinity for iron-containing substances, but this does not necessarily explain formation of ferruginous bodies by asbestos and similar substances. Examination of elements arranged by Pauling's electronegativity scale in Table I[8] shows that silicates tend to form a nucleophilic conjugate base while magnesium oxides tend to form magnesium and hyroxide ions. It is possible that an electronegative mucopolysaccharide coat acting in concert with nucleophilic silicates represents the mechan-

TABLE I
Electronegativity of Central Atoms[a]

Group:	I	II	III	IV	V	VI	VII
	Li-OH	Be-OH	B-OH	C-OH	N-OH	O-OH	F-OH
	1.0	1.5	2.0	2.5	3.0	3.5	4.0
	Na-OH	Mg-OH	Al-OH	Si-OH	P-OH	S-OH	Cl-OH
	0.9	1.2	1.5	1.8	2.1	2.5	3.0

[a] From Pauling.[8]

isms by which asbestos and other fibers attract ferric ions. Another possibility is that the coating of foreign bodies by an iron-protein attached to a mucopolysaccharide coat could be a fundamental defense mechanism related to the immune response, and asbestos and other silicate-containing minerals could act to disrupt the immune-response or foreign body reaction, producing a prolonged and continuing stress.

It is of interest to note that ferruginous body formation represents a mechanism for removal of ferric ions regardless of the nature or mechanism for formation of the ferruginous deposit. The increase in oxidative pathways most likely results in export of fibrogenic factors including lysozymes, Kreb's cycle intermediates, or polysaccharides. If encapsulation of a fiber in a mucopolysaccharide coat is involved, then a depletion of mucopolysaccharides in the macrophage cell membrane might account for its increased permeability with leaking of cellular material. It is possible to conclude that perturbation of the redox equilibrium of the phagocytic cell by removal of ferric ion to form ferruginous bodies sets the stage for successive events.

Collagen Synthesis

The synthesis and increasing tissue concentration of the various forms of collagen have been associated with chronic fibrosing

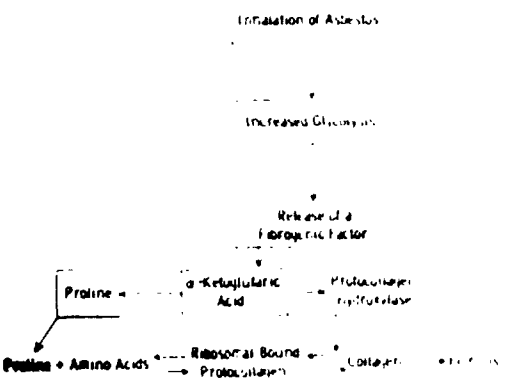

Figure 1. Induction of collagen synthesis and fibrosis by asbestos inhalation.

processes, a hypothetical description of the process is shown in Figure 1. The fibrosis resulting from asbestosis is diffuse in comparison to discrete nodular fibrosis associated with exposure to silica. The diffuse fibrosis may be related to the fact that the length of fibers precludes complete phagocytosis. Webster,[9] in studies with South African vervet monkeys exposed to two different types of airborne asbestos dusts, found that the reactions of the asbestos fibers with the body were of three distinct types: (1) an organized desquamative alveolitis; (2) an obliterative bronchiolitis; and (3) an interstitial fibrosis not directly associated with the presence of asbestos needles or bodies. The observation of an interstitial fibrosis not directly associated with the fiber supports Heppleston's hypothesis in regard to the formation and export of a fibrogenic factor by macrophage cells. Heppleston[10] contends that the factor is nonlipid in nature, possibly a protein or derivative thereof, or perhaps a galactan may be involved. Heppleston's hypothesis also suggests that the fibrogenic factor acts by stimulating hydroxyproline production by fibroblasts. However, as shown in Figure 1, hydroxyproline is formed by hydroxylation of proline residues in protocollagen, and apparently is not found as a free amino acid in the cell.[11] Thus the fibrogenic factor or factors must be intimately related to the rate-controlling step in collagen synthesis—hydroxylation of proline residues in protocollagen mediated by protocollagen hydroxylase.

Protocollagen is composed of relatively few types of amino acids, primarily proline and glycine. This results in an extremely hydrophobic molecule, and the protocollagen is thought to be strongly bound to an RNA templet. Hydroxylation of certain proline residues is carried out by reaction with molecular oxygen and a reducing agent, catalyzed by protocollagen hydroxylase. The activity of this enzyme is regulated by the level of α-ketoglutaric acid in the cell. Hydroxylation of the protocollagen forms a

water-soluble collagen that frees the RNA template for synthesis of more protocollagen, and as long as the stimulus of α-ketoglutaric acid is present, the cycle continues. The single-stranded soluble collagen forms a more stable triple-stranded collagen by cross-linking at random in solution. The triple-stranded collagen continues to form more complex collagen fibers and fibrotic bundles.[11] It is possible that lysozymes, Krebs cycle intermediates, or saccharides exported by macrophages stimulate higher levels of α-ketoglutaric acid in fibroblast cells resulting in increased rates of collagen formation. The collagen formation is also an index of synthesis for other elastic fibers, elastin and reticulin, that make up fibrin.

Carcinoma

The general view of carcinoma is one of an uncontrolled growth of cells and tissues exerting a debilitating effect eventually leading to death of the affected organism. The causes of this debilitating disease have not been definitely identified, but there is a great deal of circumstantial evidence implicating a host of environmental factors. In general, the induction of carcinoma is thought to involve interference with the flow of genetic information, particularly the information that controls cell growth. Asbestos fibers may be direct carcinogens, implying that no secondary agent is required; or they may be secondary carcinogens, promoting agents, or carriers of carcinogenic substances. Viruses, radiomimetic chemicals, toxic products of combustion, certain metals, and ionizing radiation have all been associated with an increased risk of developing cancer,[12] and it is possible that all these agents are capable of producing cancer.

Exposure to asbestos is associated with two distinct types of cancer: bronchiogenic carcinoma and mesothelioma. In bronchiogenic carcinoma, asbestos most likely acts as a secondary carcinogen or promoting agent, but in mesotheliama, the evidence strongly suggests that asbestos may act as a direct carcinogen.

To understand the mechanisms by which asbestos could exert a promoting or secondary effect, it is necessary to identify possible relationships between the biological processes associated with exposure to asbestos and the mechanisms for action of potential carcinogens. One possible mechanism is induction of changes in membrane permeability due to release of lysozymal enzymes to surrounding tissues. By modifying membrane permeability, potentially carcinogenic substances such as viruses, metals, toxic products of combustion, and carcinogenic chemicals would have easier access to critical sites in cells and tissues. This would essentially be an adverse effect on the cells' first line of defense—selective permeability of the cytoplasmic and/or nuclear membranes. A promoting effect could arise in a different fashion as a result of alterations in the major biochemical reactions in the cell, particularly in the oxidoreductase system—the main detoxification system of most cells and tissues.[11] The synthesis of collagen and related substances, elastin and reticulin, requires cofactors from this system, and there is possible inhibition of oxidoreductases by competition for cofactors required for metabolism and elimination of potential carcinogens. Such cofactor competition could explain the relationship between exposure to asbestos, smoking, and the high incidence of bronchiogenic carcinoma that has been reported among asbestos workers who smoke.[13]

An example of inhibition of the metabolism of a potential carcinogen is inhibition of electron transport in the hydroxylation of 3,4-benzopyrene. The hydroxylation of of 3,4-benzopyrene involves the consumption of one mole of molecular oxygen and two moles of hydrogen from NADPH. The hydroxylation reaction involves two distinct steps: formation of an exoperoxide–enzyme–substrate complex, and reduction of the complex by NADPH. If the cofactor for

reduction is not available, then a stable 3-4-benzopyrene exoperoxide is available for addition to DNA or other macromolecules, perhaps increasing the probability of a cancer.[14]

Asbestos has also been characterized as a potential carrier for other substances that may result in production of a cancer. Cralley[15] has postulated that certain metal contaminants on asbestos fibers may be responsible for the associated carcinogenesis, and he has shown that many of the metals associated with the fibers produce tumors. Polyaromatic hydrocarbons have also been found on asbestos fibers in trace amounts, and it is possible that these substances are introduced into tissues, exerting a tumorigenic effect. Metals or polyaromatic hydrocarbons are possibly introduced in processing, as natural contaminants in asbestos, or by absorption from the environment.

Mesothelioma is a tumor of the mesothelial tissues. Development of this tumor is poorly understood, but it seems to be highly associated with the presence of asbestos. It is possible that the mechanism for induction of mesothelioma is related to the effect of asbestos on bioenergetics of the mesothelial cells. The increase in glycolysis associated with tumor cells was described by Warburg[16] in 1930; he attributed it to an increase in operation of the respiratory chain. The idea of an increase in glycolysis being responsible for tumorigenesis was subsequently discounted,[17] but Racker [18] has revived the idea in a modified form in his hypothesis involving glycolysis and pH changes, pointing out that the increase in glycolysis is not necessarily associated with the respiratory chain. Interference with energy flow in cells and tissues can have severe consequences for the whole organism, and divalent cations, calcium and magnesium, have dramatic effects on storage and transport in mitochrondia.[19]

It is instructive to note that the magnesium content of certain types of asbestos isolated from exposed tissues is reported[20] to decrease with time, suggesting that magnesium species are leached from the fiber. The effect of magnesium ions is to change mitochondria from an orthodox (O) energy-rich form to an aggregated (A) uncoupled energy-poor form, or an O-to-A transition, while calcium ions with hormones such as thyroxine produce the opposite transition, A to O.[19] In effect, calcium ions allow mitochondria to take in and store energy, while magnesium ions cause the energy to be dissipated. The leaching of magnesium ions from asbestos fibers or fibrils in mesothelial tissues could favor development of tumor cells. It is possible that tumor cells are able to survive the challenge of magnesium ions leaching from asbestos fibers better than normal cells. In time, the normal cells are weakened or destroyed owing to energy deprivation, and the tumor cells, being more energetic, recover and begin to develop and spread rapidly in the absence of normal cells to hold them in check. Calcification of the pleural is most likely an alternate manifestation of the interplay of calcium and magnesium ions. The deposition of calcium is controlled by a delicate hormonal balance that could be upset by introduction of other divalent cations.[19]

Summary

In Figure 2, the possible relationship between exposure to asbestos, ferruginous body formation, collagen synthesis, and carcinoma is outlined. Calcification of the pleural mem-

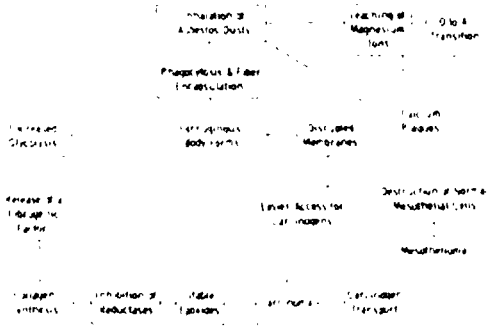

Figure 2. Possible relationship between exposure to asbestos, ferruginous body formation, collagen synthesis, and carcinoma.

branes and induction of mesothelioma are considered to be manifestations of leaching and solution of magnesium ions, but not necessarily related to other biological responses. In the biological and tissue response to inhalation of asbestos, fibers that reach the aveolar spaces are phagocytized. As a result of changes in membrane permeability caused by direct irritation of the membrane by the fiber, there is a release of cellular constituents that contain fibrogenic factor. The fibrogenic factor is possibly a protein, galactan, lysozyme, or Kreb's cycle intermediate, but in any case it stimulates production of collagen and associated elastin and reticulin by increasing glycolysis. The rate-controlling step in collagen synthesis is hydroxylation of certain proline residues in protocollagen, using molecular oxygen and a reducing cofactor. As long as the fibrogenic factor is produced, collagen synthesis continues and the asbestos may act as a secondary carcinogen by acting as a carrier for carcinogenic substances, by damaging cell membranes and increasing entry of carcinogens to cells, or by competing for reducing cofactors and inhibiting the metabolism of carcinogenic substances. The driving force for continued consumption of oxygen is to reestablish the redox equilibrium disturbed by removal of ferric-containing proteins in ferruginous body formation. It is possible that ferruginous bodies are perhaps of greater significance in environmental and occupational health than has previously been maintained.

References

1. Lehninger, A. L.: *Biochemistry*, p. 3. Worth Publishers, New York (1970).
2. Szmczykrewicz, K., and H. Wozniak: Effect of Certain Industrial Dusts on the Oxygen Uptake Capacity of the Pulmonary Tissues of White Mice. *Med. Pracy 17*:185 (1966).
3. Krebs, H. A., and R. L. Veech: Equilibrium Relations between Pyridine Nucleotides and Their Roles in the Regulation of Metabolic Processes. *Advan. Enzyme Reg. 7*:397 (1969).
4. Yatsimirskii, K. B., and V. P. Vasil'ev: *Instability Constants of Complex Compounds*, p. 70. D. Van Nostrand Co., Princeton, New Jersey (1960).
5. Gross, P., R. T. P. de Treville, L. J. Cralley, and J. M. G. Davis: *Arch. Pathol 85*:539 (1968).
6. Davis, J. M. G.: Further Observations on the Ultrastructure and Chemistry of the Formation of Asbestos Bodies. *Exp. Mol. Pathol. 13*:346 (1970).
7. Governa, M., and C. Rosanda: A Histochemical Study of the Asbestso Body Coating. *Brit. J. Ind. Med. 29*:154 (1972).
8. Pauling, L.: *The Nature of the Chemical Bond*, 3rd ed., Cornell University Press, Ithaca, New York (1960).
9. Webster, E. C.: The Pathogenesis of Asbestos. *Int. Conf. Pneumoconioses*, Republic of South Africa, Johannesburg, p. 62 (1969).
10. Heppleston, A. G.: Fibrogenic Action of Silica. *Brit. Med. Bull. 25*:282 (1969).
11. White, A., P. Handler, and E. L. Smith: *Principles of Biochemistry*, 4th ed., p. 871, McGraw-Hill, New York (1968).
12. Fishbein, C., W. G. Flamm, and H. L. Falk: *Chemical Mutagens*, Academic Press, New York (1970).
13. Selikoff, I. J., E. C. Hammond, and J. Chung: "Asbestos Exposure, Smoking, and Neoplasia. *J. Amer. Med. Ass. 204*:106 (1968).
14. Flowers, E. S.: *Aromatic Hydroxylase Inhibitors as Environmental Factors in the Production of Cancers*, Doctoral Dissertation, University of California, Berkeley (1971).
15. Cralley, L. J.: Electromotive Phenomenon in Metal and Mineral Particulate Exposure: Relevance to Exposure to Asbestos and Occurrence of Cancer. *Amer. Ind. Hyg. Ass. J. 32*: 653 (1971).
16. Warburg, O.: *The Metabolism of Tumors*, Constable, London (1930).
17. Aisenberg, A. C., and H. P. Morris: Energy Pathways of Hepatoma No. 5123. *Nature (London) 191*:1314 (1961).
18. Racker, E.: Bioenergetics and the Problems of Tumor Growth. *Amer. Sci. 60 (1)*:56 (1972).
19. Green, D. E., and J. H. Young: Energy Transduction in Membrane Systems. *Amer. Sci. 59(1)*:92 (1971).
20. Langer, A. M., I. Rubin, and I. J. Selikoff: Electron Microprobe Analysis of Asbestos Bodies. *Pneumoconiosis*, Proceedings of the International Conference, Johannesburg, Capetown, Oxford University Press (1970).