Biological in Vitro and in Vivo Responses of Chrysotile Versus Amphiboles

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Although all commercial forms of asbestos have been demonstrated to be carcinogenic in animals, so far epidemiological data are controversial concerning what asbestos types are the most carcinogenic and fibrogenic in humans. In order to understand the early cellular events induced by fibrous particles, different in vitro studies (hemolysis, release of enzymes by macrophages, assays on cell culture systems) have been carried out in several laboratories; most of these studies have shown that cell and subcellular in vitro responses were different depending on fiber types: chrysotile versus amphiboles. This presentation compares the results of different laboratories with our data obtained by using a model which modifies the chemistry of the fibers by acid treatment. The acid-leached chrysotile and acid-treated amphibole fibers showed different biological responses in several in vitro systems used in comparison to unleached fibers. These differences in the in vitro reactivity were related to the chemical state of the fibers and might explain the differences in their effects in animals after intrapleural injection as assessed by the percentage of mesothelioma, the latency period, the survival time and the degree of pleural fibrosis. The carcinogenic effect of the fibers is discussed in relation of their in vitro inflammatory or cytotoxic responses.

Introduction

Although the fibrogenic and carcinogenic properties of asbestos dusts are universally accepted, there is still considerable debate regarding on the one hand the mechanisms of fibrogenesis and carcinogenesis and on the other, the gradient in pathogenicity of different types of fibers.

With regard to the mechanisms of asbestos-related diseases, over the past 10 years considerable emphasis has been placed on the experiments of Stanton et al. (1, 2) and Pott et al. (3, 4) which demonstrated that the carcinogenic potential of fibers was mostly related to the fiber size, the most carcinogenic fibers in the pleura being those more than 4 μm in length and less than 0.25 μm in width. Actually, the Stanton hypothesis, based on the concept of the "solid state" or "foreign body" carcinogenesis, put the role of physicochemical parameters far behind (5). However, we will see later on that the role of chemical and physical constituents and particularly those available at the surface of the fibers must also play a role, but have been insufficiently assessed.

From human and animal data, there is strong evidence that the three commercial types of asbestos, the serpentine chrysotile and the amphiboles, crocidolite and amosite, are all responsible for lung and pleural fibrosis and for lung and mesothelial cancers (6). However, there is still controversy about the gradient of pathogenicity of these three types of asbestos. Several epidemiological studies on human populations which have been exposed to one type of fiber have persuaded many people that crocidolite (7-9), and perhaps also amosite (10, 11), is much more carcinogenic towards the pleura than chrysotile (12). Previously, a group of experts at the 1976 IARC meeting (6) concluded that occupational exposure to chrysotile was more likely to cause lung fibrosis and lung cancer than exposure to amphiboles. Occupational exposure to crocidolite and amosite, however, was more often associated with pleural and peritoneal mesotheliomas than exposure to chrysotile. In a recent editorial, however, Liddell (13) gave another opinion, pointing out that amphiboles were not only the most carcinogenic fibers in the mesothelium, but were also more fibrogenic and carcinogenic in the lung. Many authors are not convinced by this assertion, especially after the epidemiological demonstration by Peto that the incidence of pleural mesothelioma was almost as high in a cohort of workers ex-
posed mostly to chrysotile as in cohorts exposed only or mostly to amphiboles (14, 15).

Experiments in animals have also shown discrepancies in the fibrogenic and/or carcinogenic potential of asbestos according to the type of fiber, but in all studies other factors intervened, such as doses, mode of dust introduction (inhalation, intratracheal instillation, intraperitoneal injection, intraperitoneal injection), type of diseases induced, animal strain, age and survival time. However, the results showing a modification in the carcinogenic effects of chrysotile after acid treatment (16, 17) raised the question that other factors, besides shape and size (chemical composition, surface physicochemistry), may play a role in the induction of fibrosis and cancer.

The controversial position of scientists concerning such an important point needed a critical review in an attempt to evaluate significant information from the comparison of the biological responses of chrysotile and amphiboles in vitro as well as in vivo. The effect of acid treatment of the fibers will also be taken into account, since it can lead to a better understanding of the mechanisms of fiber carcinogenesis. Our provisional conclusions will be derived from concordant results obtained in our laboratory and in others during the last decade.

In Vitro Studies

Reactivity with Red Blood Cells

The hemolytic assay provides a rapid way for investigating the interaction between dusts and biological membranes. Using this system, several authors (18–22) found different responses with chrysotile and the amphiboles, the former being more hemolytic than the latter. Generally speaking, after acid treatment chrysotile was less hemolytic (16, 21, 22), whereas acid-treated amphiboles were found to be more hemolytic (21, 22). Thus, if hemolysis explores the interaction between fibers and cell membranes, chrysotile appeared as the most reactive fiber type in these experiments. This discrepancy was also found when studying the adsorption of phospholipids on fibers, and this was greater with chrysotile than with the amphiboles (Jaurand et al., unpublished data) (Fig. 1). Jaurand et al. (24), when studying the kinetics of hemolysis by chrysotile, have shown a self inhibition of the reaction due to adsorption of the membranes. This correlates with the observation of a decreased hemolytic activity after incubating fibers with phospholipids (24) and probably relates to a decrease in the zeta potential (26). Indeed, Light and Wei (27) have demonstrated that the hemolytic activity was related to the absolute value of the zeta potential of fibers. With chrysotile it decreased during leaching, whereas with crocidolite it increased during the same treatment.

Reactivity with Macrophages

Studies carried out in different laboratories over the last five years, describing the release of lysosomal acid hydrolases from peritoneal or alveolar macrophages maintained in culture, have clearly shown differential responses between chrysotile and the amphiboles.

Davies et al. (27) and, more recently in our laboratory, Jaurand et al. (22) have clearly shown that chrysotile works as an inflammatory stimulus, inducing a selective release of lysosomal acid hydrolases. However, there was no release of cytoplasmic enzymes such as lactate dehydrogenase (LDH), which, in the case of peritoneal macrophages, showed higher intracellular levels, suggesting an enhanced protein synthesis (27). Similar responses have been obtained with other particles such as zymosan which we know to elicit inflammation (28).

This type of inflammatory response could be related to the physicochemical surface properties of the fibers, since acid-treated chrysotile, which has lost most of its Mg, did not release lysosomal enzymes (28) and even released LDH, indicating a cytotoxic effect (22, 29).

In contrast, untreated amphiboles (crocidolite and amosite) seemed to be cytotoxic, releasing both lysosomal acid hydrolases and cytoplasmic LDH. Acid-treated amosite and crocidolite, however, enhanced the release of lysosomal hydrolases, but this was associated with the release of LDH (22) (Fig. 2).
CHRYSTOTILE VS. AMPHIBOLES

It has also become clear that macrophages secrete a number of products (enzymes, mediators) after incubation in vitro with asbestos and that several of these molecules may be directly involved in chronic inflammatory responses. Chrysotile has been shown to elicit a highly significant increase in macrophage phospholipase activity and prostaglandin synthesis (30) and to induce the secretion by alveolar macrophages, of a chemotactic factor which attracts polymorphonuclear leukocytes (31). Macrophages from mice given intraperitoneal injections of chrysotile asbestos secrete considerable amounts of plasminogen activator when cultured in vitro, whereas latex particles do not yield such an increase (32). All these in vitro studies indicate clearly that chrysotile works as a very potent activating factor on alveolar macrophages. However, other studies did not confirm the greater stimulatory effect of chrysotile. Thus, White and Kuhn (33) found an increased secretion of elastase by peritoneal macrophages under the action of chrysotile and crocidolite, but the high doses used in this experiment do not allow a comparison of the effect. Moreover, in a recent experiment exploring the oxidant production by guinea pig alveolar macrophages in vitro (release of O₂⁺ and H₂O₂) an opposite pattern was shown, e.g., the amphibole asbestos were more effective than chrysotile to cause macrophage oxidase activation (34). Other in vitro experiments are needed, taking into account most of the parameters involved (animal species, types of macrophage, culture conditions and so on). This will allow us to better understand the significance of the in vitro responses of macrophages in different biological pathways according to fiber types, in relation to the type and intensity of fibrogenesis and carcinogenesis in vivo: however the relationship between the in vitro macrophage response to fibers and pulmonary or pleural carcinogenesis, however, is far from being clearly understood.

Reactivity with Proliferative Cells

Several authors have used cell lines in short-term studies of asbestos cytotoxicity. Most of these studies are summarized in Table 1 which indicates the cell lines used by the authors, the cytotoxicity assays, the doses of asbestos tested and the gradient of toxicity according to the type (chrysotile versus amphiboles) of fiber (35-41). In most studies chrysotile was more “toxic” than the amphiboles. Moreover, the acid treatment decreased the cytotoxicity of chrysotile and increased the cytotoxicity of crocidolite and amosite (41).

Up to now, few experiments have been conducted with normal tissue or cells in culture. Some of them were carried out with normal tracheal tissue explants cultured in vitro (42). Only amphiboles have
Chinese Rat
Chinese Adult
Human intestine-derived
Chinese hamster ovary CHO cells
Chinese hamster lung V 79-4 cells
Chinese alveolar lung A 549 cells
Chinese hamster lung-derived CHL 39 cells
Chinese hamster ovary CHO cells
Human intestine-derived I 407 cells
Adult rat liver-derived ARL 6 cells
Mouse colon-derived MCE 1 cells

Epithelial-like cell lines | Cytotoxicity assays | Asbestos doses, µg/mL | Gradient toxicity* | Reference
---|---|---|---|---
Macrophage-like P 388 D1 cells | Growth inhibition | 10-100 | Ch > Cr | (35, 36)
Human lung fibroblasts W 38 cells | Morpho changes | 100 | Ch > Am | (37)
Rat liver-derived K 22 cells | Growth inhibition | 10 | Ch >> Cr > Am | (37)
Chinese hamster ovary CHO cells | Colony efficiency | | | 
Chinese hamster lung V 79-4 cells | Colony efficiency | 10-50 | Am > Cr > Ch | (38)
Chinese alveolar lung A 549 cells | Growth inhibition | | Ch > Cr > glass fibers > LCh | 
Chinese hamster lung-derived CHL 39 cells | Colony efficiency | 10 | Ch > Am >> Cr | (39)
Chinese hamster ovary CHO cells | Growth inhibition | 10 | Ch > Cr, Am | (40)
Human intestine-derived I 407 cells | Colony efficiency | 250 | Ch 10 times > Am-Cr | 
Adult rat liver-derived ARL 6 cells | Colony efficiency | | | 
Mouse colon-derived MCE 1 cells | Colony efficiency | | | 

*Ch = chrysotile; Cr = crocidolite; Am = amosite; LCh, LCr, LAm = leached chrysotile, crocidolite, amosite.

been tested in this model. In our laboratory, we have developed a model using cultures of normal rat mesothelial cells for testing the reactivity with different types of fiber (43). This test studied the morphology and the growth characteristics of mesothelial cells treated with chrysotile and crocidolite which were either oxalic-acid leached or unleached (44). When the samples are compared weight to weight, the results agree with those obtained by others who used epithelial-like cell lines (37, 41). Thus, chrysotile seems to be more reactive and cytotoxic with epithelial-like cell lines than crocidolite; leaching of chrysotile fibers decreased the reactivity; conversely, leaching the crocidolite increased the cytotoxic effects on the cells.

In vitro studies have also been carried out with cultures of lung fibroblasts which were stimulated to produce fibrous collagen under the action of different types of asbestiform minerals (45). In these experiments, chrysotile was the most reactive, followed by anthophyllite and amosite/crocidolite. This effect was dose-dependent, but the response was not constantly the same. In contrast, the acid-leached chrysotile, particularly when 80% of the magnesium was depleted, was much less active on collagen synthesis.

Subcellular Effects

It is still controversial as to whether or not asbestos can bind to DNA and induce damage and mutations. No mutagenicity was demonstrated by means of the Ames tests on bacteria (46). However, tests carried out on mammalian cells in culture have shown that asbestos fibers may interact with DNA, since they gave a weak mutagenic response with the HGPRT mutant phenotypic test (39), induced chromosomal damage (45) and slightly increased sister chromatid exchanges (38). However, no difference was noted between chrysotile and the amphiboles.

Although it has been demonstrated that chrysotile asbestos was much more active than the amphiboles in binding IgG (48), no difference was noted between chrysotile and the amphiboles for the activation of the classical and alternative pathways of complement (48, 49). Apparently, complement activation was not related to reactive sites at the fiber surface, since there was no difference between chrysotile and the amphiboles or between chrysotile and leached chrysotile (49, 50).

Nevertheless, surface properties seem important for the adsorption of macromolecules by asbestos fibers, as suggested by the results obtained in our laboratory with chrysotile and oxalic acid-leached chrysotile. The adsorption of albumin or dipalmitoyl phosphatidyl choline on Mg-depleted chrysotile fibers was characterized by a bulk incorporation of the macromolecules into the fibers. However, these results are different from those of others (51, 52) who found that albumin had a decreased affinity for Mg-depleted chrysotile.

Animal Studies

Differential Fibrogenesis

The early animal experiments did not clearly define the relative importance of asbestos fiber types in the production of lung or pleural fibrosis (53, 54). However, since the work of Wagner et al. (55) and more recently of Davis et al. (56), it appears that
Chrysotile given by inhalation causes far more lung fibrosis than crocidolite, which in turn is more fibrogenic than amosite. This fibrogenic gradient was still found to be the same when the number of fibers was adjusted to an equivalent number in dust samples (56). This gradient may be related to fiber size discrepancies between asbestos types as suggested by many authors. Davis et al. (56), who used an extensive fiber-length distribution, showed that the chrysotile clouds in the chamber had many more fibers over 20 μm in length than either of the amphibole clouds in their experiment. It seems that short fibers, less than 5 μm in length, are phagocytosed without causing fibrosis, while fibers longer than 5 μm in produce foreign body granuloma with fibrosis. In a recent, well-controlled animal experiment using inhalation, Lee et al. (57) found that amosite was at least 10 times more fibrogenic than potassium octatitanate (Fybex) fibers, although concentrations and lengths of these man-made organic fibers were many times higher in the clouds than those of amosite. These findings suggest that physicochemical properties of the surface of the fibers must play an important role in fibrogenesis.

Differential Carcinogenesis

Several experiments, some of them large-scale, have been carried out in different species in order to study the differential effect of fibers introduced into the pleural or peritoneal cavities, either by injection or by implantation. The intrapleural or intraperitoneal inoculation of dusts has the advantage that experiments can be conducted with small amounts of material which allow the comparison of various samples of specially prepared or modified dusts. However, the experiments using the inhalation of dusts through the airways are more realistic when compared with human exposure: the ideal is chronic inhalation in a special chamber.

Most early animal inhalation studies did not find differential results in the production of bronchial carcinomas and mesotheliomas with different asbestos types (58-60). Wagner et al. (55), in a series of experiments in rats using amosite, anthophyllite, crocidolite and two varieties of chrysotile, found that the shortest mean survival time after first exposure was observed with chrysotile, particularly the Canadian one, followed by crocidolite and amosite. In the same way, the highest number of malignant tumors was observed in animals treated with Rhodesian chrysotile and the lowest in those treated with amosite. Anthophyllite, crocidolite and Canadian chrysotile gave about the same number of tumors. The more carcinogenic effects of chrysotile versus the amphiboles were observed even though much less dust was retained in the lungs exposed to chrysotile. Davis et al. (56) found clear-cut results after inhalation studies in rats comparing UICC chrysotile A, crocidolite and amosite. UICC chrysotile A was more fibrogenic and carcinogenic than UICC crocidolite and UICC amosite, since all the malignant lung tumors were found in animals that had inhaled chrysotile dust. Only two mesotheliomas were found in this study, one with crocidolite, and one with chrysotile.

Regarding pleural carcinogenesis, it also appears that chrysotile is the most carcinogenic—or at least as carcinogenic as the amphiboles. As early as 1969, Wagner and Berry (61), in a large-scale experiment comparing the effect of chrysotile, crocidolite and amosite on specific pathogen-free (SPF) and standard rats, found clear-cut results, in that all types of asbestos produced mesotheliomas. Chrysotile and crocidolite produced about the same percentage of tumors, the percentage in SPF animals with mesotheliomas being 61% for chrysotile and 59% for crocidolite, while in standard animals the corresponding percentages were 69% and 68%. The fewest mesotheliomas were produced by amosite (40% of the SPF and 31% of the standard animals). Moreover, when comparing the mean survival times for SPF and standard rats with mesotheliomas, after eliminating the effect of mortality due to other causes, the authors found that chrysotile exposure led to the shortest survival times (598 days for SPF and 621 days for standard rats). This was significantly less than crocidolite (718 and 655, respectively) and much less than amosite (811 and 801 days, respectively). In contrast, the survival of SPF and standard rats without mesothelioma, after elimination of the effect of mortality due to mesothelioma, was not different. Wagner et al. (55), however, using intrapleural inoculation of various dusts in rats, found that among the UICC standard reference samples (experiment 3), UICC crocidolite was the most carcinogenic, being three times as active as UICC chrysotile. But, in this very paper, the results of experiment 1, where SFA chrysotile was compared to crocidolite in a dose-effect relationship, were in contradiction with the above conclusion. There was a relationship between the number of mesotheliomas and the dose (from 0.5 to 8 mg) for both SFA chrysotile and crocidolite, but if we total the number of rats with a mesothelioma, there were 21 out of 59 animals with mesotheliomas in the SFA chrysotile group while there were 11 out of 59 animals with mesotheliomas in the crocidolite group.

In a recent study carried out in our laboratory, after intrapleural injection of different dusts in the rat, chrysotile and crocidolite produced about the
same number of mesotheliomas; the most striking difference was a more marked initial inflammatory reaction of the pleura with chrysotile, with a greater percentage of animals dead from other causes than cancer. Moreover, the latency period was shorter in the chrysotile group than in the crocidolite group (17).

**Conclusion**

Obvious discrepancies exist between the biological effects of chrysotile and the amphiboles either in vitro or in vivo. Chrysotile seems to be the most reactive in vitro as well as in vivo. These findings question whether or not it is scientifically correct to apply the Stanton theory generally to carcinogenesis induced by fibers, since it takes into account only the fiber size parameters, length and diameter. In this respect, it is odd that the paper in memory of Stanton (62) takes into account only amphiboles and amphibole-like fibers, excluding chrysotile fibers, which according to our results and to those of other laboratories, appear as the most potent inflammatory stimulus. The striking modification in the biological response of acid-treated asbestos suggests that reactive sites at the surface of the fibers could also play a role in the pathogenic effects of fibers, particularly in relation to cancer. Thus the difference in the survival time between chrysotile and crocidolite in rats whose pleural cavity had been injected with fibers might be due to the fact that chrysotile was immediately reactive in inducing inflammation and subsequently cancer, whereas crocidolite needed some in vivo modification to become inflammatory and carcinogenic.

This puzzling biological problem makes the interpretation of human data difficult. First, humans have usually been exposed to mixed fibers associated with different cofactors. Peto et al. (14), analyzing epidemiological data, observed that the risk of developing mesothelioma was substantially lower in humans whose exposure to chrysotile was reduced or ceased than in those where exposure was maintained; by contrast, even brief exposure to crocidolite could produce a substantial incidence of mesothelioma many years later (6). Peto et al. (14) suggests that this difference could be due either to the fact that chrysotile was largely eliminated from the lung whereas amphiboles remained almost indefinitely (63) or to the fact that chrysotile fibers are leached in vivo (64) and thus cease to be biologically active in the body, while crocidolite fibers remain active or even become more active.

Recent experiments in animals have shown that other fibrous minerals such as erionite-zeolite were also carcinogenic, even more than asbestos, although fibers were short (65). This underlines the necessity of pursuing basic research on the mechanisms of the biological effect of fibers because it seems that the Stanton hypothesis does not explain all situations.

**REFERENCES**

1. Stanton, M. F., and Wrench, C. Mechanisms of mesothelioma induction with asbestos and fibrous glass. J. Natl. Cancer Inst. 48: 797-821 (1972).
2. Stanton, M. F., Layard, M., Togeris, A. Miller, E., May, M., and Kent, E. Carcinogenicity of fibrous glass: pleural response in the rat in relation to fiber dimension. J. Natl. Cancer Inst. 58: 587-603 (1977).
3. Pott, F., Huth, F., and Friedrichs, K. H. Tumorigenic effect of fibrous dusts in experimental animals. Environ. Health Perspect. 9: 313-315 (1974).
4. Pott, F., Friedrichs, K. H., and Huth, F. Results of animal experiments concerning the carcinogenic effects of fibrous dusts and their interpretation with regard to the carcinogenesis in humans. Zbl. Bakt. Hyg. I. Abt. Orig. 162: 467-509 (1978).
5. Harington, J. S. Fiber carcinogenesis: epidemiological observations and the Stanton hypothesis. J. Natl. Cancer Inst. 67: 977-987 (1981).
6. IARC. Asbestos (Monographs on the Evaluation of Carcinogenic Risk to Chemicals to Man, Vol. 14), International Agency for Research on Cancer, Lyon, 1977.
7. Wagner, J. C., Sleggs, C. A., and Marchand, P. Diffuse mesothelioma and asbestos exposure in the North Western Cape Province. Brit. J. Ind. Med. 17: 260-271 (1960).
8. Jones, J. S. P., Pooley, F. D., and Smith, P. G. Factory populations exposed to crocidolite asbestos. A continuing survey. INSERM 52: 117-120 (1978).
9. McDonald, A. D., and McDonald, J. C. Mesothelioma after crocidolite exposure during gas mask manufacture. Environ. Res. 17: 340-346 (1978).
10. Acheson, E. D., Gardner, M. J., Bennett, C., and Winter, P. D. Mesothelioma in a factory using amosite and chrysotile asbestos. Lancet: 1403-1406 (1981).
11. McCullagh, S. F. Amsite as a cause of lung cancer and mesothelioma in humans. J. Soc. Occup. Med. 30: 153-156 (1980).
12. McDonald, J. C., Liddell, F. D. K., Gibbs, G. W., Eysen, S. E., and McDonald, A. D. Dust exposure and mortality in chrysotile mining 1910-75. Brit. J. Ind. Med. 37: 11-24 (1980).
13. Liddell, D. Asbestos and public health. Thorax 36: 241-244 (1981).
14. Peto, J. The incidence of pleural mesothelioma in chrysotile asbestos textile workers. In: Biological Effects of Mineral Fibers, Vol. 2, No. 30 J. C. Wagner, Ed.), International Agency for Research on Cancer, Lyon, 1980, pp. 703-711.
15. Peto, J., Seidman, H., and Selikoff, I. J. Mesothelioma mortality in asbestos workers: implications for models of carcinogenesis and risk assessment. Brit. J. Cancer 45: 124-135 (1982).
16. Morgan, A., Davies, P., Wagner, J. C., Berry, G., and Holmes, A. The biological effects of magnesium-leached chrysotile asbestos. Brit. J. Exptl. Pathol. 58: 465-473 (1977).
17. Monchaux, G., Bignon, J., Jaurand, M. C., Lafuma, J., Sebastien, P., Masse, R., Hirsch, A., and Goni, J. Mesotheliomas in rats following inoculation with acid-
leached chrysotile asbestos and other mineral fibres. Carcinogenesis 2: 229-236 (1981).

18. Schnitzer, R. J., and Pundsack, F. L. Asbestos hemolysis. Environ. Res. 3: 1-13 (1970).

19. Harington, J. S., Miller, K., and Maenab, G. Hemolysis by asbestos. Environ. Res. 4: 95-117 (1971).

20. Rahman, Q., Narang, S., Kaw, J. L., and Zaidi, S. H. Asbestos induced haemolysis in relation to its silica solubility. Environ. Physiol. Biochem. 4: 284-288 (1974).

21. Light, W. G., and Wei, E. T. Surface charge and asbestos toxicity. Nature 265: 537-539 (1977).

22. Jaurand, M. C., Magne, L., Boumlier, J. L., and Bignon, J. In vitro activity of alveolar macrophages and red blood cells with asbestos fibres treated with oxalic acid, sulfur dioxide and benzo-3,4-pyrene. Toxicology 21: 323-342 (1981).

23. Jaurand, M. C., Thomassin, J. H., Baililif, P., Magne, L., Touray, J. C., and Bignon, J. Chemical and photoelectron spectrometry analysis of the adsorption of phospholipid model membranes and red blood cell membranes to chrysotile fibres. Brit. J. Ind. Med. 37: 169-174 (1980).

24. Jaurand, M. C., Magne, L., and Bignon, J. Inhibition by phospholipids of haemolytic action of asbestos. Brit. J. Ind. Med. 36: 113-116 (1979).

25. Jaurand, M. C., Renier, A., and Bignon, J. The adsorption of phospholipids and red blood cell membranes on chrysotile fibres. In: The In Vitro Effects of Mineral Dusts (R. C. Brown, M. Chamberlain, R. Davies and I. P. Gormley, Eds.), Academic Press, New York, 1980, pp. 121-124.

26. Light, W. G., and Wei, E. T. Surface charge and hemolytic activity of asbestos. Environ. Res. 13: 135-145 (1977).

27. Davies, P., Allison, A. C., Ackerman, J., Butterfield, A., and Williams, S. Asbestos induces selective release of lysosomal enzymes from mononuclear phagocytes. Nature 251: 423-425 (1974).

28. Schorlemmer, H. U., Davies, P., Hylton, W., Gugig, M., and Allison, A. C. The selective release of lysosomal acid hydrolase from mouse peritoneal macrophages by stimuli of chronic inflammation. Brit. J. Exptl. Pathol. 58: 315-326 (1977).

29. Beck, E. G., Holt, P. F., and Nasrrallah, E. T. Effects of chrysotile and acid-treated chrysotile on macrophage cultures. Brit. J. Ind. Med. 28: 179-185 (1971).

30. Sirois, P., Rol-Plesczynski, M., and Begin, R. Phospholipase A activity and prostaglandin release from alveolar macrophages exposed to asbestos. Prostagl. Med. 5: 31-37 (1980).

31. Schoenberger, C., Hunninghake, G., Gadek, J., and Crystal, R. G. Role of alveolar macrophages asbestos exposure: modulation of neutrophil migration to the lung following asbestos exposure. Amer. Rev. Resp. Dis. 121 (suppl): 257 (1980).

32. Hamilton, J., Vassalli, J. D., and Reich, E. Macrophage plasminogen activator: induction by asbestos is blocked by anti-inflammatory steroids. J. Exptl. Med. 144: 1699-1694 (1976).

33. White, R., and Kuhn, C. Effects of phagocytosis of mineral dusts on elastase secretion by alveolar and peritoneal exudative macrophages. Arch. Environ. Health 35: 106-109 (1980).

34. Hatch, G. E., Gardner, D. E., and Menzel, D. B. Stimulation of oxidant production in alveolar macrophages by pollutant and latex particles. Environ. Res. 23: 121-136 (1980).

35. Wade, M. J., Lipkin, L. E., Tucker, R. W., and Frank, A. L. Asbestos cytotoxicity in a long-term macrophage-like cell culture. Nature 264: 444-446 (1976).

36. Wade, M. J., Lipkin, L. E., and Frank, A. L. Studies of in vitro asbestosis-cell interaction. J. Environ. Pathol. Toxicol. 2: 1029-1039 (1979).

37. Neugut, A. I., Eisenberg, P., Silverstein, M., Pulkrabek, P., and Weinstein, J. B. Effects of asbestos on epithelioid cell lines. Environ. Res. 17: 259-265 (1979).

38. Chamberlain, M., and Brown, R. C. The cytotoxic effects of asbestos and other mineral dust in tissue culture cell lines. Brit. J. Exptl. Pathol. 59: 183-189 (1978).

39. Huang, S. L. Amonite, chrysotile and crocidolite asbestos are mutagenic in Chinese hamster lung cells. Mutat. Res. 68: 265-274 (1979).

40. Livingstone, G. K., Rom, W. N., and Morris, M. V. Asbestos-induced sister chromatid exchange in cultured Chinese hamster ovarian fibroblasts. J. Environ. Pathol. Toxicol. 3: 373-382 (1980).

41. Reiss, B., Solomon, S., Weisburger, J. H., and Williams, G. M. Comparative toxicities of different forms of asbestos in a cell culture assay. Environ. Res. 22: 109-129 (1980).

42. Mossman, B. T., Kessler, J. B., Ley, B. W., and Craighead, J. E. Interaction of crocidolite asbestos with hamster respiratory mucosa in organ culture. Lab. Invest. 36: 131-139 (1977).

43. Jaurand, M. C., Bernaudin, J. F., Renier, A., Kaplan, H., and Bignon, J. Rat pleural mesothelial cells in culture. In Vitro 17: 98-106 (1981).

44. Jaurand, M. C., Bastie-Sigeac, L., Renier, A., and Bignon, J. Comparative toxicities of different forms of asbestos on rat pleural mesothelial cells. Environ. Health Perspect. 51: 153-158 (1983).

45. Hext, P. M., and Richards, R. J. Biochemical effects of asbestos minerals on lung fibroblast cultures. Brit. J. Exptl. Pathol. 57: 281-285 (1976).

46. Chamberlain, M., and Tarnay, E. M. Asbestos and glass fibers in bacterial mutation tests. Mutat. Res. 43: 159-164 (1977).

47. Sincock, A., and Seabright, M. Induction of chromosome changes in Chinese hamster cells by exposure to asbestos fibres. Nature 257: 56-58 (1975).

48. Hasselbacher, P. Binding of immunoglobulin and activation of complement by asbestos fibers. J. Allerg. Clin. Immunol. 64: 294-298 (1979).

49. Wilson, M. R., Gaumer, H. R., and Salvaggio, J. E. Activation of the alternative complement pathway and generation of chemotactic factors by asbestos. J. Allerg. Clin. Immunol. 60: 218-222 (1977).

50. Saint Remy, J. M. R., and Cole, P. Interactions of chrysotile asbestos fibres with the complement system. Immunology 41: 431-437 (1978).

51. Morgan, A. Adsorption of human serum albumin by asbestos fibres and its application to the measurement of surface areas of dispersed samples of chrysotile. Environ. Res. 7: 330-341 (1974).

52. Valerio, F., Veggi, M., and Santi, L. Adsorption isotherms of albumin and ferritin on Rhodesian chrysotile. Environ. Res. 21: 186-189 (1980).

53. Holt, P. F., Mills, J., and Young, D. K. Experimental asbestosis in the guinea-pig. J. Pathol. Bacteriol. 192: 185-195 (1966).

54. Wagner, J. C., and Skidmore, J. W. Asbestos dust deposition and retention in rats. Ann. N.Y. Acad. Sci. 132: 77-86 (1965).

55. Wagner, J. C., Berry, G., Skidmore, J. W., and Timbrell, V. The effects of the inhalation of asbestos in rats. Brit. J. Cancer 29: 252-269 (1974).

56. Davis, J. M. G., Beckett, S. T., Bolton, R. E., Collings, P., and Middleton, P. Mass and number of fibres in the pathogenesis of asbestos-related lung disease in rats. Brit. J.
57. Lee, K. P., Barras, C. E., Griffith, F. D., Waritz, R. S., and Lapin, C. A. Comparative pulmonary responses to inhaled inorganic fibers with asbestos and fiberglass. Environ. Res. 24: 167-191 (1981).

58. Gross, P., and de Treville, R. T. P. Experimental asbestosis. Arch. Environ. Health 15: 638-649 (1967).

59. Reeves, A. L., Puro, H. E., Smith, R. G., and Vorwald, A. J. Experimental asbestos carcinogenesis. Environ. Res. 4: 496-511 (1971).

60. Reeves, A. L., Puro, H. E., and Smith, R. G. Inhalation carcinogenesis from various forms of asbestos. Environ. Res. 8: 178-202 (1974).

61. Wagner, J. C., and Berry, G. Mesotheliomas in rats following inoculation with asbestos. Brit. J. Cancer 23: 567-581 (1969).

62. Stanton, M. F., Layard, M., Tegeris, A., Miller, E., May, M., Morgan, E., and Smith, A. Relation of particle dimension to carcinogenicity in amphibole asbestos and other fibrous minerals. J. Natl. Cancer Inst. 67: 965-975 (1981).

63. Bignon, J., Sebastien, P., Gaudichet, A., and Bonnau, G. Measurement of asbestos retention in humans related to health effects. In: Workshop on Asbestos: Definitions and Measurement Methods (C. C. Gravatt, P. D. Lafleur, and K. F. J. Heinrich, Eds.), NBS Special Publication 506, Washington, DC, 1978, pp. 95-119.

64. Jaurand, M. C., Bignon, J., Sebastien, P., and Goni, J. Leaching of chrysotile asbestos in human lungs. Environ. Res. 14: 245-254 (1977).

65. Wagner, J. C. Health hazards of substitutes. Paper presented at Symposium on Asbestos, Montreal, May 24-27, 1982.