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SECTION VII. ASBESTOS AND NEOPLASIA: EXPERIMENTAL

STUDIES OF CARCINOGENESIS OF ASBESTOS FIBERS AND THEIR NATURAL OILS*

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The mode of action of asbestos in producing asbestosis and neoplasia is at present little understood. Pulmonary fibrosis, the main feature of asbestosis, has been ascribed to mechanical or chemical factors, or to a combination of both; the mechanism of carcinogenic action is almost completely unknown.

Two forms of neoplasia in man are associated with exposure to asbestos: bronchogenic carcinoma[1] and diffuse pleural mesothelioma.[2]

THE TYPES OF ASBESTOS INVOLVED IN MALIGNANCY IN MAN

"Asbestos" is the generic name given to a class of fibrous mineral silicates which may vary considerably in physical and chemical composition. In some studies in the past the term asbestos has been used without proper definition and the type of material used remains unidentified.

A priori, the demonstration of differences in the carcinogenic activity between different types of asbestos, either in epidemiological or experimental studies, would open the way to a critical investigation of asbestos carcinogenesis.[3]

Studies of mortality by district of residence[4] have suggested that exposure to crocidolite asbestos in the North-Western Cape carries with it a distinct risk of developing malignant disease. In the case of the crocidolite mines in the North-Western Cape, asbestos mining districts showed a three-fold excess of deaths from lung cancer as compared with adjoining districts, and as compared with control districts of similar population and degree of urbanization. This latter comparison is important since in South Africa, as elsewhere, lung cancer mortality is much lower in rural than urban districts. The excess was seen in both males and females.

Similar figures for cancer mortality in the eastern and north-eastern areas, where chrysotile and amosite asbestos are mined and milled revealed no excess mortality from any form of cancer. In these regions, however, the relative proportion of the population exposed to the asbestos is considerably reduced by comparison with that in the North-Western Cape. Although the standardized mortality ratios might suggest that exposure to

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crocidolite is a greater cancer hazard for man than exposure to amosite and chrysotile, it would be premature to decide until further investigations of the populations actually exposed have been carried out.

For example, Roe and Walters[5] recently pointed out that tobacco smoking is so strongly associated with lung cancer that it has to be taken into account in all studies of possible associations between the disease and other factors. In Britain there is a 45-fold difference in lung cancer incidence between nonsmokers and heavy cigarette smokers.[6] By comparison the three-fold difference observed by Oettlé[4] is small.

Oettlé's work was based on certification of death and involved no matching with regard to smoking habits nor exposure to many of the other known carcinogens involved in the etiology of lung cancer. The figures therefore, can be regarded as confirmatory only. At least they indicate that even before the risk of mesothelioma became widely recognized, an increased mortality from cancer of the lung was being recorded in the asbestos mining districts of the North-Western Cape.

TABLE 1*
PRODUCTION OF ASBESTOS (IN THOUSANDS OF TONS) FROM THE NORTH-WESTERN CAPE AND TRANSVAAL AREAS IN SOUTH AFRICA

Years	Transvaal		North-Western Cape
	amosite	crocidolite	crocidolite
1928-1935	35	0.1	31
1936-1943	118	13	49
1944-1951	237	48	85
1952-1959	446	126	345

*From Keep, 1961.[7]

Oettlé also suggested that asbestos might not have been mined on a sufficiently large scale for a sufficiently long time for an excessive lung mortality to be apparent in the Transvaal amosite (and crocidolite) mining areas. However, production figures for the period 1928–1959 (TABLE 1) show that there is little difference between the production of amosite in the North-Eastern Transvaal and crocidolite in the North-Western Cape.

Wagner, Sleggs and Marchand,[2] in a study of individual cases, observed an association between pleural mesothelioma and exposure, either occupational or nonoccupational (that is, in persons living close to asbestos mines or mills), to crocidolite asbestos in the North-Western Cape. On the other hand, no cases have been observed in populations exposed to amosite only.[4,8-9] Sluis-Cremer[8] has dealt in this monograph with some of the geo-

graphical and environmental considerations which apply to the apparent difference in carcinogenicity in man of these two types of closely-related amphibole asbestos. He has reported that none of the factors examined explains satisfactorily the absence of pleural mesothelioma in the Transvaal. These include mineralogical differences between the two areas, intensity of production and the extent of environmental pollution and asbestosis. Recently, in North America, primary peritoneal tumors have been found in workers exposed to chrysotile.[9,10]

Previous Experimental Studies

Sarcomata have been produced in rats after the subcutaneous and intraperitoneal injection of asbestos of an undisclosed type.[11] The fibers were longer (0.5 to 1.0 cm.) than those used in other studies. Two other silicates, augite and tremolite, failed to produce tumors under the same conditions. Augite belongs to the pyroxene group of minerals, the most important group of rock-forming ferromagnesian silicates,[12] and has the formula $(Ca, Mg, Fe^{2+}, Al)_2 (Si, Al)_2 O_6$. Tremolite is an amphibole mineral, commonly of fibrous nature and with the formula $Ca_2 Mg_5 [Si_{18} O_{22}] (OH, F)_2$.

Wagner, in 1962,[13] induced pleural mesotheliomata by inoculating various dusts directly into the pleural cavity of rats. Pleural tumors were found in two animals inoculated with crocidolite fibers, one with chrysotile, and one with finely-divided silica powder (99.9 per cent SiO_2). A rat exposed to a chrysotile dust cloud also developed a pleural mesothelioma. No tumors developed in rats which had received carbon black. Experiments at present in progress and reported by Skidmore and Wagner elsewhere in this monograph,[14] show that crocidolite, chrysotile, silica powder and to a lesser extent, amosite, all induce pleural mesotheliomata in guinea pigs after intrapleural injection. Malignant tumors have been produced in fowls by crocidolite and amosite,[15] though it is too early to determine their relative carcinogenicity in this species.

Possible Modes of Action of Asbestos as a Carcinogen

At present the following possible mechanisms of carcinogenesis are under consideration:

(1) That carcinogenesis is due entirely, or in part, to organic materials associated with the fiber, either naturally or as a result of contamination or treatment of the asbestos during processing;

(2) that it is due to the presence of certain carcinogenic metals or metal-complexes in asbestos;

(3) that it is an "Oppenheimer Effect," that is to say, that it is induced by the prolonged residence in the tissues of chemically-inert material incapable of being rapidly removed by phagocytosis.

Oils and Other Organic Material Associated with Asbestos

Three types of asbestos are used industrially: crocidolite, amosite and chrysotile. The first two of these, but not the third, contain appreciable amounts of natural oils which may be removed to a large extent by extraction with suitable solvents.[16,17] Crocidolite, amosite and chrysotile may also contain secondary oils as a result of the addition of oil to the asbestos during processing, or as a result of contamination. Asbestos is normally transported and stored in jute bags and the oil from the jute fibers is absorbed to an appreciable extent (70 to 80 per cent) by all three types of fiber. Jute bags are almost always used in the industry, from the point where the fiber leaves the mill to the point where the manufacturer uses it for his own purposes. In processing, rebagging may be done and there paper-lined bags or plastic may be used, the latter being costly.

An account of the natural (primary) and secondary oils which may be found in asbestos has been given by Harington elsewhere in this monograph.[17]

The Natural Oils

As reported elsewhere,[17] it is not yet known whether there are any quantitative or qualitative differences between crocidolite and amosite oils, nor whether these oils vary in yield and composition from batch to batch, or within the same sample of fiber. On the other hand, there seems little doubt that finely-divided asbestos yields more oil than the longer fibers in milled material.

Large amounts of crocidolite and amosite oils (150 to 200 ml.) have been extracted from 45 kg. amounts of mixtures of both finely-divided and commercial fiber, using double-distilled petroleum ether. It is important to record that the solvent used for these extractions was of a high degree of purity. Ultra-violet and infra-red spectra indicated that it was apparently free of contaminating materials and no residue was detected when a two-liter sample was evaporated to dryness. The yields for both oils were approximately 0.3 per cent by weight of the crude asbestos fiber before extraction.

The two oils differed in color and in infra-red spectra, and more polycyclic hydrocarbons were detected in amosite than in crocidolite. A sample of the crocidolite oil was found to contain 1 μg. benzo[a]pyrene/100 g. fiber and the amosite, 5 μg. benzo[a]pyrene/100 g. fiber. Details of these analyses are given by Harington elsewhere in this Annal.[17]

The concentrations of benzo[a]pyrene and related polycyclic hydrocarbons are so low that it is difficult to believe that their presence plays more than a minor carcinogenic role, even in persons exposed most heavily to crocidolite or amosite dusts. The elution by serum of benzo[a]pyrene and such hydrocarbons from asbestos is probably equally insignificant.

Biological Tests for Carcinogenicity

In order to assess the carcinogenic role of natural asbestos oils and other extractable material, the following experiments have been undertaken:

(a) *Tests of crude and extracted asbestos for carcinogenicity in subcutaneous tissues of rats and mice.*

These tests are based on the assumption that tumors are likely to arise at the sites of subcutaneous injection of native asbestos fibers in these two species. If this assumption is justified, a comparison of the relative carcinogenicity of the exhaustively-extracted fibers with that of the fibers before extraction, would help to define the role of the extractable material in carcinogenesis by asbestos.

In controlled experiments, rats and mice have been injected with saline suspensions of (1) the native fibers of crocidolite, amosite and chrysotile; (2) the fibers of crocidolite and amosite following successive extraction with 8 different solvents (see reference 17 for procedure).

Tests, using BALB/C mice, have been in progress for 10 months, and those using CBA mice, for only 15 weeks. In BALB/C mice which have died from intercurrent disease, sizeable deposits of asbestos have been visible at the injection sites in all groups. Microscopically, some of the asbestos fibers, especially the smaller ones, were seen to lie within phagocytes. Others were still extracellular. Lymph glands draining the injection area contained asbestos-laden phagocytes. Similarly-laden phagocytes were not detected by ordinary microscopic methods in other tissues. Fibrosis has not been seen in relation to the injection sites in any of the asbestos-treated BALB/C mice, but is already apparent on palpation in some of the CBAs, especially in the groups treated with chrysotile. In the cases of amosite and crocidolite, where fibrosis is less frequent, there is no difference in incidence between groups treated with crude and extracted samples. So far, there have been no injection-site tumors in either mouse strain. It is emphasized, however, that the experiments are incomplete.

(b) *Tests of a sample of amosite from a factory where cases of lung cancer had been reported.*

After the association between exposure to asbestos and mesothelioma[2] had been recognized, attention was drawn to the occurrence of four cases of lung cancer in persons apparently exposed to amosite asbestos in a factory in the United States.[18] By good fortune there remained for investigation a large consignment of the actual asbestos fiber to which the patients had been exposed. Samples of this fiber have been the subject of chemical and biological tests, both in the laboratory of Smith[19] and in our laboratory.

In the biological tests undertaken by us, the carcinogenic activity of (1) native amosite fiber and (2) amosite extracted with a series of solvents[17]

are being compared. Rats have been injected intrapleurally with 25-mg. amounts of each sample. The experiment is now in the 10th week.

(c) *Tests for carcinogenicity and cocarcinogenicity on mouse skin.*

(1) *Asbestos Oils.* Experiments have been started in which the oils obtained by mass extraction of crocidolite and amosite with petroleum ether are applied repeatedly to the skins of mice. These experiments are still only at a very early stage. In preliminary tests the oils (either undiluted or as 50 per cent or 25 per cent dilutions in acetone) were found to be non-irritant to mouse skin, causing no apparent epidermal hyperplasia and no dermal changes. Carcinogens of the polycyclic hydrocarbon type produce characteristic changes in mouse skin when applied in high concentration.[20,21] The fact that these changes were not seen in our preliminary tests indicates that compounds of this type are probably not present in the oils in high concentration, and this has been confirmed by chemical analysis.[17] For this reason a strong carcinogenic response is not to be expected.

Though not strongly carcinogenic, it is possible that the asbestos oils possess incomplete carcinogenic activity of either the tumor-initiating or tumor-promoting type. The lack of strong irritant activity is unlikely to be associated with potent promoting activity. But the possibility that the oils are effective initiators of the carcinogenic process remains entirely open. Our tests for incomplete carcinogenic activity consist of (*a*) a repeated application of the oils to mouse skin following a single initiating dose of 7,12-dimethylbenz[a]anthracene (DMBA) and (*b*) a short course of applications of the oils followed by repeated applications of croton oil.

(2) *Jute Oils.* Heavy contamination of asbestos with jute oils would only be of significance in relation to carcinogenesis if the oils themselves were shown to have relevant biological activity. Accordingly, samples of each of two types of oil used in jute manufacture were tested for carcinogenicity and tumor-promoting activity on mouse skin.

Groups of 20 male mice of the Chester Beatty Stock Strain were clipped and treated as follows: *Group 1*: 150 μg. 7,12-dimethylbenz[a]anthracene (DMBA) in acetone once when the mice were 6 weeks old, followed, after a five-week treatment-free interval, by twice-weekly applications of undiluted light mineral oil for 20 weeks. *Group 2*: Same as Group 1 except that "soluble" oil was given as the second treatment. *Group 3*: received DMBA in the same way as Groups 1 and 2, but no secondary treatment. *Groups 4 and 5*: received no primary treatment with DMBA, but from the age of 11 weeks onwards they were treated with light mineral oil and "soluble" oil, respectively, in the same way as Groups 1 and 2.

The 150 μg. dose of DMBA given to Groups 1, 2 and 3 was intended to be sufficient to initiate tumor formation without completing the process. In fact, during an observation period of 45 weeks after application of DMBA, only one skin papilloma arose in the mice of Group 3 which re-

TABLE 2
TESTS FOR CARCINOGENICITY AND TUMOR-PROMOTING ACTIVITY OF JUTE OILS

Group	Number of mice	Primary treatment (once only)	Secondary treatment (0.25 ml × 2 weekly) for 20 weeks.†	Tumor development after 20 weeks of secondary treatment.			
				Surviving mice	Mice with skin tumors	Total papillomata	Total carcinomata
1	20	150 μg. DMBA*	Undiluted light material oil used in jute manufacture.	20	13	118	8
2	20	150 μg. DMBA*	Undiluted "soluble" oil used in jute manufacture.	18	1	1	0
3	20	150 μg. DMBA*	NONE	19	1	1	0
4	20	NONE	Undiluted light mineral oil used in jute manufacture.	17	3	4	0
5	20	NONE	Undiluted "soluble" oil used in jute manufacture.	18	0	0	0

*7, 12-Dimethylbenz[a]anthracene.

† Secondary treatment was begun 5 weeks after application of DMBA.

ceived no other treatment. Mice of Group 1, on the other hand, developed 118 benign and 8 malignant skin tumors during the 20-week period of secondary treatment with light mineral oil after DMBA. This observation indicates that the sample of oil used has considerable tumor-promoting activity. The "soluble" oil showed no such activity.

Groups 4 and 5 represented tests for complete carcinogenicity of the two oils. By the 20th week of the experiment three out of 17 survivors of Group 4, treated with light mineral oil only, had developed a total of four benign papillomata. Mice of Group 5 were still without skin tumors at this time. Although no malignant tumors were seen, these results indicate that the light mineral oil probably possesses weak carcinogenic activity.

Taken together, these findings, which are summarized in TABLE 2, suggest that the storage of asbestos in jute bags may introduce a significant variable into subsequent determinations of its carcinogenic activity.

Metal Carcinogenesis and Asbestos

Many metals have been shown to be capable of inducing cancer.[22] Among the more notable ones are arsenic, chromium, nickel, cobalt, cadmium, lead and beryllium. In addition, several other metals, though not apparently carcinogenic in the simple ionic state, became so when combined in certain macromolecular complexes. The best-known example of this is iron, which as ferrous sulfate or gluconate shows no carcinogenic activity but which, in the forms of iron-dextran, iron-dextrin, saccharated iron oxide or ferrous glutamate, is markedly active.[23] Aluminum is another example in so far as aluminum-dextran is carcinogenic.[23]

Iron is in fact one of the more interesting metals in relation to asbestos (*vide infra*). Hematite, which contains 70 per cent of iron, all in the ferric state, has been suspected of inducing cancer of the lung in man.[24] However, the basis on which this suspicion was founded has recently been questioned.[25] Wagner[13] reported the induction of pleural mesotheliomata in rats injected with silica powder and this has been confirmed.[14] A sample of the powder used in these experiments was examined for iron and a value of approximately 0.06 per cent was obtained. Of this, 0.04 per cent was in the form of ferric oxide (Fe_2O_3) and 0.02 per cent as ferrous oxide (FeO), giving a Fe^{3+} : Fe^{2+} ratio of 1 : 0.6. The values for iron are low and do not support the suggestion that the iron content of the silica powder plays the same kind of role in carcinogenesis as it does in iron-dextran. On the other hand, it is possible that the presence of iron was essential to the carcinogenic activity of the silica.

Small amounts of iron are known to catalyze certain reactions of biological interest.[26-27] Silica powder not freed of its associated metals (0.6 mg. iron/gm. silica powder and 0.0016 mg. copper/gm. powder and trace amounts of aluminum and other metals)[27] totally oxidized glutathione in *in vitro*

conditions, but silica with a greatly reduced metal content after acid-washing had no effect.[27] The oxidation process appears to be due to the iron present in the silica, since similarly low concentrations of either ferric and ferrous iron are capable of oxidizing glutathione to the same extent.

Against the background of these considerations it is interesting to consider the metal contents of the three types of asbestos.

The amphibole asbestos types, crocidolite and amosite, are fibrous in nature and are complex silicates containing magnesium, iron, calcium and sodium in varying proportions. Crocidolite ("Cape blue asbestos") is a sodium ferroso-ferrisilicate with a composition approximating to $Na_2(Fe^{2+}, Mg^{2+})_3 (Fe^{3+})_2 Si_8 O_{22} (OH)_2$ and consists of about 50 per cent silica and 40 per cent iron (as oxides), fairly equally distributed as ferric and ferrous forms. The ratio of Fe^{3+} : Fe^{2+} is 1 : 1.4.

Amosite, which is closely related to crocidolite chemically, is found exclusively in South Africa. It is a ferrous magnesium silicate and contains the same amount of total iron as crocidolite but mostly in the ferrous form. The Fe^{3+} : Fe^{2+} ratio is 1:91. The formula approximates to $(Mg^{2+}, Fe^{2+})_7 Si_8 O_{22} (OH)_2$.

The serpentine asbestos, chrysotile, is a magnesium silicate, fibrous in nature and of formula $Mg_3 Si_2 O_5 (OH)_4$. It consists of 40 per cent silica and 40 per cent magnesium (as oxides) and relatively small amounts of iron (2.5 per cent as oxides), made up of ferrous or ferric iron replacing magnesium in the structure, and partly of magnetite impurities. The Fe^{3+} : Fe^{2+} ratio is 1:1.3.

With regard to the oxidation of glutathione, the three types of asbestos proved to be hardly less effective than unwashed silica powder.[27] At *p*H 7.4 the following amounts of the dust oxidized 100 μg. glutathione: chrysotile 6.6 mg., silica powder 50 mg., crocidolite 66.6 mg. and amosite 133 mg. Chrysotile totally destroyed glutathione, the reaction proceeding beyond oxidation.

Quite apart from the absolute amounts of iron present, the ratio of ferric to ferrous iron may be important. Such data as is available *suggests* that carcinogenicity may be associated with a *low* ferric: ferrous ratio (see TABLE 3). In order to determine whether this association is valid, a large number of minerals with known contents of ferric and ferrous iron are being tested in mice by subcutaneous injection.

Two other interesting metallic constituents of asbestos are nickel and chromium since it is beyond dispute that both these metals cause lung cancer.[28-29] Both may occur in relatively high concentration (e.g. 0.5 per cent Ni and 0.1 per cent Cr) in chrysotile.[17]

To summarize the position with regard to metals in asbestos: in the cases of crocidolite and amosite the iron content is well in excess of that in certain macromolecular complexes of iron, in particular iron-dextran, known

TABLE 3
POSSIBLE RELATION OF THE RATIO OF FERRIC AND FERROUS IRON IN VARIOUS MINERALS WITH THEIR CARCINOGENICITY

Mineral	Fe^{3+} : Fe^{2+}	Carcinogenicity	Reference
crocidolite	1:1.4	+	2,4,13-14
chrysotile	1:1.3	+	4,13-14,19
silica	1:0.6	+	13-14
amosite	1:91	±	4,13-14,19
augite	1:4†	-	11
tremolite	1:8.6†	-	11
anthophyllite	1:1.7*	unknown	—
	1:9.6†		

* mean of three values for Finnish anthophyllite, determined here.
† values from reference 12: mean of eleven values for anthophyllite, of ten for augite and of five for tremolite. All other analyses from reference 17.

to be highly carcinogenic in animals. The iron content of chrysotile, though much lower, may still be sufficient to account for carcinogenesis by a mechanism comparable to that of iron-dextran. Silica powder with a much lower content of iron has been shown to be carcinogenic in animals; in this case iron may be playing a different, though nevertheless essential, part in the carcinogenic process. The second mechanism may apply to asbestos also. Finally, the possibility that the ferric: ferrous ratio rather than the absolute content of the iron is important in relation to carcinogenesis requires further study. Of the other metals in asbestos, the high nickel and chromium contents of chrysotile deserve the most attention.

Experiments in Progress

Clearly these theoretical considerations provide the basis for many critical animal experiments. Because of limitation of facilities a start to these has only recently been made. In the first experiment the carcinogenicity of (1) native crocidolite, amosite and chrysotile asbestos, and (2) acid-washed asbestos[17] of the same three types, is being compared by the method of subcutaneous injection in mice. Acid washing removes large amounts of metals from asbestos without apparently affecting the structural integrity of the fibers, as shown by X-ray diffraction analysis of the fibers.

ASBESTOS CARCINOGENESIS AS AN EXAMPLE OF TUMOR-INDUCTION BY CHEMICALLY INERT MATERIALS

In agreement with Schmähl,[11] we feel it is unlikely that there is much in common between the mechanism by which asbestos induces cancer and

the so-called "Oppenheimer Effect." In the first place, native asbestos cannot be regarded as chemically inert in the same way as many of the materials studied by the Oppenheimers and their colleagues, and secondly, the essential characteristic of the Oppenheimer Effect is that carcinogenesis only occurs when large bodies are implanted — no effect being seen when the same materials are introduced in powdered form.

Summary

Recent epidemiological studies on lung cancer and mesothelioma have revitalized the subject of the mechanism of carcinogenesis by asbestos. In a parallel paper,[17] the chemistry of the three main types of asbestos is discussed. In this paper possible mechanisms of carcinogenesis, and experimental methods of elucidating them, are considered from a biological standpoint. The significance of oils naturally present in asbestos and of oils purposely or accidentally added to it, and the possible role of metal constituents, are discussed in some detail. It is regarded as unlikely that asbestos carcinogenesis is an example of the Oppenheimer Effect.

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