PLAINTIFF'S EXHIBIT

DOW-1452

144

ST0065517

Ingested Mineral Fibers

Do They Penetrate Tissue or Cause Cancer?

Paul Gross, MD; Russell A. Harley, MD; Layinka Margaret Swinburne, MRCP, MRC Path; John M. G. Davis, PhD; William B. Greene

Three different laboratories independently investigated the ability of ingested mineral fibers to penetrate tissues in rats. All concluded that there was no evidence of tissue penetration by ingested mineral fibers.

These experimental observations are supported by the findings in coal and hard-rock miners who swallow, during their lifetime, nearly 100 times the amount of dust that is stored in their lungs. The intestinal wall or mesenteric lymph nodes of these people show no evidence of storage of the ingested dust particles. Animals fed asbestos over much of their lifetime and allowed to live to the age of cancer production, failed to provide evidence of a cancerogenic effect.

Submitted for publication May 31, 1974; accepted Aug 9, 1974.

From the Medical University of South Carolina, Charleston (Drs. Gross, Harley, and Mr. Greene), St. James's Hospital, Leeds, England (Dr. Swinburne), and the Institute of Occupational Medicine, Edinburgh (Dr. Davis).

Reprint requests to the Department of Pathology, Medical University of South Carolina, 80 Barre St, Charleston, SC 29401 (Dr. Gross).

Because of the association of peritoneal mesotheliomas with asbestos exposure, it was suggested that inhaled asbestos fibers cleared from the lungs and swallowed, migrated through the gastrointestinal (GI) wall to the peritoneum there to initiate the cancer. The more recent discovery of submicronic mineral fibers in beers, wines, and drinking water has resulted in publicized expressions of fear that a health hazard exists.

A recent investigation suggests that the lungs of city dwellers not occupationally exposed to asbestos may contain many millions of submicron-sized fibers. A percentage of these fibers have been identified as chrysotile. Amphibole asbestos fibers have also been identified in the lungs of city dwellers but their amount or concentration has not been determined.[2]

Since only about 1% to 2% of the dust inhaled during a lifetime is retained in the lungs, the other 98% to 99% being transported via the pulmonary clearance mechanism to the pharynx,[3] it is probable that the GI tract will be the recipient of much of the inhaled and subsequently ingested dust particles, nonfibrous as well as fibrous.

Ingestion of asbestos fibers has undoubtedly been in progress in some countries for centuries, inasmuch as fibers of submicronic dimensions occur naturally in river water as it erodes serpentine and amphibole outcroppings. Increased industrial applications of mineral fibers may have accelerated this ingestion somewhat. Nevertheless, the world literature does not suggest that an alarming increase in GI inflammatory disease or cancer has occurred, or that the incidence is higher in regions where asbestos fibers are present in natural waters.

Because of the publicly expressed fears that ingestion of beverages or food containing submicronic mineral fibers may expose large populations to a cancer-producing hazard, responsible public officials are demanding that the scientific community confirm or refute the assertion that a health hazard exists. The present report is intended to be a response to this demand. A portion of the work here reported was begun nine years ago, long before the possible consequences of asbestos ingestion became of widespread interest. However, because of their negative nature, the results were not published. Two other laboratories more recently did publish negative results of asbestos feeding studies: one, as an abstract or short note[4]; and the other, as part of a discussion on the dangers of nitrites in food.[5]

When it was learned recently that three laboratories were either engaged in a study on asbestos ingestion or had completed it, agreement was reached to pool the results of these studies in the present joint paper.

Taconite tailings from an iron mine, containing submicronic mineral fibers have been piped into Lake Superior for nearly 20 years. Because some ultramicroscopic mineral fibers have been discovered recently in the drinking water from this lake, dust from this mineral is included in these studies.

MATERIALS AND METHODS

Chrysotile Feeding

Ground laboratory chow was intimately mixed with ball-milled chrysotile asbestos (consisting of the whole spectrum of fiber lengths and diameters) so that the latter constituted 5% by weight of the mix. This was supplied along with water ad libitum to ten male rats for 21 months at which time all were killed. There was no time period between the asbestos feeding and death to allow the GI tract to rid itself of the ingested asbestos. Five laboratory control rats of the same age as the asbestos-fed animals were killed at the same time as the latter.

The GI tract of the animals was carefully examined for lesions and random pieces of the tissue were removed for paraffin impregnation and subsequent sectioning. Blocks of tissue were also removed from the thoracic and other abdominal organs. The remainder of these tissues was placed together in a jar of formaldehyde solution for possible future study. Because no thought was given at that time to the possibility of transmigration of fibers, tissue from the GI tract was not isolated from other tissues and no attempt was made to keep the formaldehyde solution that had been contaminated by asbestos-containing fecal material from contaminating the other tissues with asbestos fibers.

Paraffin sections, cut at 6μm and stained with hematoxylin-eosin, were made of the various tissues. One-millimeter blocks were cut from the fixed tissue of the GI tract after nine years sojourn in formaldehyde solution and embedded in epoxy resin for electron microscopic examination. Weighed portions of the large and small intestines, and of the mesentery as well as of the other organs were processed as described below for determination of their mineral fiber content.

Administration of Fibrous Minerals by Gavage

Amosite and taconite mine tailings were finely ground in a mill (Spex) so that an aqueous suspension of either dust could be ejected through an 18-gauge needle. Unanesthetized rats were given 1 ml of suspension containing 400 mg of the dust by gavage, using an 18-gauge blunted needle. Because some of the animals struggled during the gavaging, a certain amount of trauma occurred. Ten rats were given amosite and ten, taconite. Five rats of the same age were left untreated as controls. Two gavaged rats from each series and one control animal were killed at 24 and 48 hours, and one, two, and three weeks later.

At autopsy, the GI tract was carefully removed so that the remaining tissues did not become contaminated with the content of the GI tract. Weighed portions of the various tissues were processed in a manner similar to that of the preceding study and according to the method to be described.

Oleomargarine Feeding

Because of the likelihood of traumatic hematogenous introduction of mineral fibers during the gavaging—a possibility recognized belatedly—a new series of animals was initiated. For this series, finely ground amosite and taconite tailings were each intimately incorporated into oleomargarine to obtain a 10% amosite content or a 20% taconite content. Rats were housed in individual cages (ten for each mineral) and deprived of all food except for 50 gm of the dust-containing oleo. During this time (six days), the dust consumption per rat ranged from 2.2 gm to 5 gm amosite and from 6.0 to 10 gm taconite. Ten control rats received no dust.

One, two, three, four, and five weeks after completion of the dust-feeding period, two rats from each series were killed. Two control rats were killed at the third, fourth, and fifth week, and one at the first week (three other control rats had died during the night). At autopsy, weighed portions of the GI tract and of other organs were processed according to the method described below. Blocks of intestinal wall fixed in formaldehyde, 1 mm in diameter, were removed for electron microscopic study.

Butter Feeding

In the second laboratory, three experiments were conducted: a preliminary short-term study, and two long-term experiments. The animals were SPF rats of the Wistar strain, initially about 12 weeks old. The asbestos was sieved to a fiber length up to 100μm and was incorporated in butter so as to result in either a 0.4% or 0.2% mixture.

1. In the preliminary study, a small number of rats were given a single dose of either 20 or 40 mg crocidolite asbestos. Fecal pellets were examined by optical microscopy and after monatomic ashing at 39.7 mm Hg, at 200 w, from between 15 minutes to one hour, at intervals from six hours to one week after feeding. The animals were killed from between 1 week to 19 days and were dissected so as to avoid contamination by gut contents. The gut and other tissues were fixed in formaldehyde solution. Paraffin sections (6μm thick) were examined by optical microscopy and samples of tissue were also subjected to monatomic ashing as described for fecal pellets.

2. Groups of rats were fed according to the following schedules once weekly for 16 weeks: 31 rats—10 mg Rhodesian chrysotile; 33 rats—5 mg crocidolite of unknown origin; and 34 rats—10 mg crocidolite of the same unknown origin. On the same schedule a control group of 24 rats was given the butter only. All rats were fed a normal diet of standard pelleted food in addition.

3. A group of 35 rats was fed once weekly for 15 weeks 10 mg of North West Cape crocidolite, and another group of 37 rats received 10 mg of Transvaal crocidolite on the same schedule. There was also a butter-fed control group of 21 rats. All these rats were also additionally fed a normal diet of standard pelleted food.

A few animals from each group of each experiment were killed in the first few months to assess any possible intermediate pathological changes in the abdominal region or elsewhere. The remainder were allowed to survive until they became moribund or died a natural death. At autopsy, any exudate present in the body cavities was aspirated and examined microscopically. Where no exudate was present, the cavities were washed out with a small volume of normal saline and also examined for the presence of cells or asbestos fibers. The lungs were inflated in situ with formaldehyde saline and removed intact.

All tissues suspected of neoplastic change were examined histologically. In addition, the major organs, abdominal wall, and chest wall, and lymph nodes were examined in a proportion of animals from each batch (53 in all). Samples of this tissue were also subjected to monatomic ashing to facilitate the recognition of asbestos residues.

Long-Term ad Libitum Feeding of Asbestos in Butter

These studies, in the third laboratory, on the penetration and transmigration of ingested asbestos fibers have not been com-

ST0065518

...d but the data that have been obtained on a few animals are included here because they complement and supplement the information obtained by the other two laboratories.

Chrysotile and crocidolite, properly ground, were intimately mixed with butter to make two batches, each containing 5 mg/gm of butter of one or the other type of asbestos. Two groups of 12 rats each were fed one or the other type of asbestos ad libitum. Animals were killed one and three months after the start of ingestion and sections of the esophagus, stomach, small intestine, cecum, colon, mesenteric lymph nodes, liver, spleen, and lung were prepared for light microscopy and transmission electron microscopy.

Preparation of Tissue Digests (First Laboratory).—Weighed amounts of tissue generally between 1 and 5 gm were added to about 20 ml of 5% sodium hypochlorite (commercial bleach) and allowed to be digested. In order to accelerate the digesting process, the mixture was placed in an oven at 50 to 60 C. It often became necessary to add new hypochlorite solution to the tissue in order to carry the process to completion. When a clear solution was obtained, it was centrifuged and the sediment washed three times with large amounts of unfiltered tap water. Care was taken during the washing so as not to lose any sediment. The latter was finally made up to a volume of 1 ml with distilled water and 20µl of the well-mixed suspension was placed on an electron microscope grid. Photographs were taken of all structures that had a greater than 1:3 aspect ratio.

[illegible] of Electron Microscope [illegible] (First Laboratory).—Some preparations initially were not satisfactory and resulted in enigmas that were resolved when structures that had been interpreted as fibers by light microscopy were found to be needle-like crystals. These readily dissolved and disappeared when the suspension was acidified.

Digestion of Tissue vs Tissue Section: Relative Sensitivity of Methods.—Considering the weight of tissue in a section on an electron microscope grid to be about 1.5×10^{-3} mg and the amount of digested mesenteric tissue represented in a 20µl drop on an electron microscope grid to average about 28 mg, then the latter grid represents nearly 19,000 times as much tissue as the grid containing a section of tissue 1-mm square and 1µm thick. In other words, if only one fiber were found on a grid containing a digest of mesenteric tissue, it would have been necessary to examine approximately 19,000 sections in order to find that one fiber in the same tissue.

Although the digestion method is capable of sampling amounts of tissue many thousand times greater than can reasonably be sampled with the method using sections 1-mm square and 1µm thick, it also has the disadvantage of being susceptible to give false-positive results. These may be caused by incomplete digestion of tissues giving rise to fibers in approximately the same size range of those ingested, but distinguishable from the latter in other respects. Another, more troublesome, cause of false-positive results is contamination. The contamination could theoretically be avoided by careful technique during the autopsy of the animal, the removal and weighing of the tissues, and by conducting the digestion and the preparation of the digests as well as of the grids in a clean room supplied with filtered air. It would also be necessary to use only water and reagents that had been passed through a Millipore filter.

RESULTS

Chrysotile-Fed Rats (First Laboratory)

Although they had ingested a large amount of asbestos each day for almost two thirds of their lifetime, the rats had thrived and appeared healthy. There were no deaths in this group. At autopsy, no lesion could be found by gross examination in the GI tract of these animals. Random paraffin sections of the GI tract likewise revealed no important abnormality microscopically. Sections of the other organs showed no evidence of damage. As might have been expected, the digests of the tissues of the GI tract that included the mucosa and its covering material, when examined with the electron microscope, contained chrysotile fibers. However, no fibers were found in the sections of intestines examined with the electron microscope.

What appeared at first glance to be of considerable importance was the finding of fibers in the digests of mesenteric tissue of three rats. In one, a chrysotile fiber was found. In the other two, the fibers were nonchrysotile. In a fourth rat, two nonchrysotile fibers were identified in a digest of a mixture composed of cardiac, pulmonary, renal, and splenic tissues.

However, two control rats were also found to have chrysotile fibers in the tissue digest of the GI tract. One of these two controls also had a nonchrysotile fiber in the same digest. All fibers were, of course, submicronic in dimensions.

Asbestos and Taconite Dosage by Gavage (First Laboratory)

One rat, gavaged with amosite and killed 48 hours later was found to have a lacerated and perforated esophagus. Much of the amosite was extravasated into the paravertebral muscles in the form of a streak parallel to the esophagus. The digests of the mesentery, kidney, and lung of this animal contained fibers consistent with amosite as well as other fibers. This is the only rat out of eight gavaged with amosite and examined that showed fibers in the digest of tissues outside of the GI tract. (Two other rats gavaged with amosite had died overnight and were discarded.) In only one out of ten rats gavaged with taconite were fibers found in a digest of tissues other than those of the GI tract. In the digest of lung tissue from this animal, three fibers were found.

Six of eight rats given amosite showed fibers in the digest of GI tract tissues and similar findings were made in the digests of the GI tract tissues of two out of ten rats given taconite tailings. No digest of tissue from the GI tract of rats killed after the 48-hour period contained fibers. A chrysotile fiber was found in the digest of GI tissue of a rat gavaged with taconite tailings.

Of the five control rats killed with this series of gavaged animals, one was discarded because it died during the night. The digest of mesenteric tissue of one control contained two fibers. A fiber was also found in the digest of kidney from another control rat.

Feeding With Oleomargarine (First Laboratory)

The digests of the GI tract of all animals fed with margarine contained a few fibers that were consistent with those incorporated in the oleomargarine. A chrysotile fiber was found in the digest of a rat fed amosite.

A single fiber was found in the di-

ST0065519

gest of mesenteric tissue of one taconite tailings-fed rat. On the other hand, the digest of the mesentery of an amosite-fed rat contained a chrysotile fiber and in two other amosite-fed rats the digests of mesenteric tissue contained two fibers each. A fiber with the splayed end typical of asbestos was found in the digest of lung tissue from a rat fed amosite and killed two weeks later. The digests of the tissues of the other animals inclusive of those of the controls contained no fibers. Electron microscopic study of the sections of intestinal wall revealed no fibers. The findings so far enumerated are summarized in Table 1. It should be emphasized that all of the fibers found were of ultramicroscopic dimensions.

Butter Feeding (Second Laboratory)

Preliminary Experiment.—After a single dose of crocidolite, light microscopic examination showed abundant fibers in fecal pellets after 24 hours. Examination after monatomic ashing showed smaller numbers of fibers in 48-hour pellets; but thereafter none were detected by either method. Examination of the gut and other organs showed no fibers even after a 40 mg dose.

Long-term Experiments.—Study with the light microscope of the tissues of the animals as well as of the aspirates and washings of the serous cavities, revealed no evidence of transmigration of asbestos fibers. No mesothelioma was encountered in these animals. Although two breast cancers and one lymphoma of axillary nodes were noted in one asbestos-fed group, similar cancers were seen in the control group, three breast cancers, one lymphoma, and one sarcoma of the thigh (Table 2). No lesions of any kind were seen that could be related to the ingested asbestos.

Long-Term ad Libitum Feeding of Asbestos in Butter (Third Laboratory)

Light microscopic examination of all tissues of animals killed one and three months after the start of asbestos ingestion showed no asbestos fibers that had penetrated into the tissues. Electron microscopic examination of the small intestine and mesenteric lymph nodes is complete for the animals killed after one month, and far advanced for the rats killed after three months. No sign of penetration by asbestos fibers has been seen.

COMMENT

Chrysotile Feeding

Inasmuch as the rats in this study had been on the asbestos diet up to the time of death, the surface of the gut could be expected to be covered by a fiber-containing film of mucin. These fibers would and did become manifest in the digests of these tissues.

Although the presence of the fibers in the other tissues could conceivably be explained on the basis of transmigration, a more probable explanation would be that the surface of these tissues became contaminated with fibers while in the same jar with the cut segments of the GI tract.

The presence of fibers in the tissues of control animals can be explained only on the basis of several embarrassing circumstances. These are concerned with contamination: one, from the ambient air; another could have occurred when the technician was weighing the tissues. The weighing pan could have been covered by fiber-containing moisture derived from the GI tract of a previous weighing. A third source of contamination could have been the unfiltered water and reagents used in the preparation of the digests.

The following conclusions can safely be drawn from these results:

1. Twenty-one months of contact with high concentrations of chrysotile fibers caused no gross or microscopic lesion of the intestinal wall. Inasmuch as lung cancers[5] and other chest cancers[7] induced by asbestos in rats have made their appearance from 16 to 17 months after the first exposure, this 21-month exposure to extremely high dosage of asbestos dust should have been adequate for a cancerogenic action to become manifest if such a potential were present in this mode of asbestos exposure.

2. Electron microscopic examination of the gut wall disclosed no evidence of transmigration of fibers.

3. Although the method of examining the sediment of digested tissues for the presence of fibers allows for the scanning of the equivalent of a volume of tissue many thousand times greater than can be examined on an electron microscope grid, the former has not yielded incontrovertible proof that transmigration of fibers occurs. Only one chrysotile fiber was found in the digests of tissues other than those of the GI tract and this, in only one animal out of ten. Furthermore, the importance of this finding becomes doubtful in view of the fact that tissue digests of two control rats also were found to contain chrysotile fibers, and in the tissue digest of one of these controls, a nonchrysotile fiber was found.

Administration of Fibrous Minerals by Gavage

The widespread dissemination of amosite fibers in tissues outside of the GI tract of the rat in which the perforation of the esophagus and extravasation of amosite into paravertebral muscles occurred is reminiscent of the experiment of Pontefract and Cunningham.[7] These writers performed laparotomies on rats and injected asbestos dust through the gastric wall. Here, the needle tore through tissues, opening vessels in the path of the needle. Furthermore, the needle track, remaining open for a time, allowed leakage of fibers into the abdominal cavity and onto the cut edges of the laparotomy. When these workers concluded that asbestos fibers transmigrated from the GI tract to practically all tissues of the body inclusive of blood, they ignored the artifacts inherent in their technique.

It is surprising that, with the potentially traumatic technique of gavaging, only one of 18 rats gave evidence of the widespread dissemination of fibers characteristic of the Pontefract and Cunningham technique and that in another gavaged rat, three fibers were found in the digest of lung tissue.

In spite of the indication that false-positive evidence of transmigration could have been obtained (as indi-

ST0065520

cated by the presence of a chrysotile fiber in the digest of GI tissue of a rat given taconite tailings, and the presence of a fiber consistent with either amosite or taconite tailings in the digest of mesenteric tissue of a control rat), it is noteworthy that following the administration of a single large dose (400 mg) of either amosite or taconite, no evidence of transmigration of fibers was observed in 16 of the 18 rats gavaged.

Oleomargarine Feeding

The results of this portion of the investigation indicate that if transmigration of mineral fibers from the GI tract occurs at all, it must be a rare event since only five fibers consistent with those ingested were found in the digests of mesentery of three rats and four such fibers were found in the lung digests of two other rats (Table 1). Other fibers (five in all) found in the digests of mesenteric tissue of three rats probably represented contaminations—such as the chrysotile fiber in the digest of the rat fed amosite.

The importance of the nine fibers (Table 1) found in the tissue digests of five of the 37 rats fed or gavaged with mineral fibers must be evaluated in the perspective of the billions or billions of fibers that had been introduced into the GI tract of these animals. If transmigration of fibers through the GI wall were a fact, one would logically expect more than one to three fibers out of many billions to accomplish this feat, and this phenomenon should be demonstrable in more than five out of 37 rats. The dose of asbestos ingested by the rats is equivalent to a consumption of 1.1 to 2.5 kg asbestos by a 75-kg man in six days, and double this amount in terms of taconite tailings. The importance of the nine fibers found is reduced to a negligible level by the finding in four rats of five fibers that are obvious contaminants, thus pointing to a very high probability that the nine fibers in question are also contaminants. As indicated previously, all fibers were ultramicroscopic in dimensions, and could have been derived from the air of the laboratory, as well as from the unfiltered water and the technique used (weighing of the tissues) in the preparation of the study samples.

Butter Feeding

The failure to detect penetration of the GI mucosa or transmigration of optically visible fibers in this study is important since asbestos fibers in this size range are known to be pathogenic, and there is a growing body of evidence to indicate that asbestos fibers in the submicronic size range are not pathogenic.[9] The fact that no cancer of the GI tract or mesothelium developed after long-term exposure of the GI mucosa to large numbers of asbestos fibers is highly significant.

Long-Term ad Libitum Feeding of Asbestos in Butter

The lack of success in the third laboratory using optical as well as electron microscopy in finding evidence of penetration of asbestos fibers after one and three months of ingestion of these mineral fibers respectively, adds weight to the significance of these negative findings in the other two laboratories.

GENERAL COMMENTS

The method of digest[illegible] in this investigation by electron microscopy is faulted by the possibility of fiber contamination that may be difficult to evaluate and thereby give rise to false-positive results. The possibility of false-negative results also exists. For instance, fibers may be lost by incomplete digestion of the tissue, by incomplete sedimentation during centrifugation, and by being covered with partially digested material on the electron microscope grid. However, in view of the ratio of the amount of tissue examined by the digestion method as compared with that examined by the electron microscope section method (many thousands to one), the chance of a negative result with the former when the latter is positive would appear to be negligible.

Although transmigration of large objects such as bullets from one portion of the body to another has been documented, this transmigration is concerned with foreign materials that had already achieved penetration into the body interstitium. The penetration of inhaled dust particles through the alveolar membrane and the subsequent lymphatic transport to satellite nodes has been recognized and studied experimentally for more than a century but the mechanism by which this penetration takes place is still unknown. Drinker and Field determine that particles may penetrate the alveolar membrane and appear in the lymphatic fluid within minutes after their insufflation into the lung of a dog. This rapid penetration of the alveolar membrane

Table 1.—Tabulation of Fibers Found in Digests of Tissues*

Tissue Digest	Dust	Fibers Found: No.	Fibers Found: Week-Rat†	Contamination Fibers (Chrysotile and Other): No.	Contamination Fibers (Chrysotile and Other)
Mesentery	Amosite 7 rats	2	2 wk	1	1 wk (chrysotile)
		2	5 wk		
	Taconite tailings 10 rats	1	1 wk	1	2 wk (chrysotile) GI tract
	Control (no dust) 4 rats			2	
Lung or kidney	Amosite 10 rats	1	2 wk (lung)		
	Taconite tailings 10 rats	3	2/7 wk gavage (lung)		
	Control (no dust) 7 rats			1	(kidney)
Total	48 rats	9		5	

* Electron microscopic examination.
† Week-rat refers to the animal killed at specified time.

ST0065521

Table 2.—Tabulation of Tumors Found in Rats Fed Asbestos

Types of Tumor	Experiment 2				Experiment 3		
	Chrysotile, 10 mg	Crocidolite, 5 mg	Crocidolite, 10 mg	Control	NW.Cape Crocidolite, 10 mg	Transvaal Crocidolite, 10 mg	Control
Mesothelioma	0	0	0	0	0	0	0
Carcinoma	2 (breast)	0	0	3 (breast)	0	0	0
Sarcoma	0	0	0	1 (thigh)	0	0	0
Lymphoma	0	0	1 (axillary nodes)	1	0	0	0
No. of animals	31	33	34	24	35	28	22

particles was confirmed by Hamburger and Robertson[11] using a different technique.

There are, however, important differences between the alveolar membrane that protects the lung interstitium from intrusion by foreign material and the GI mucosa that, among other functions, also performs a similar guard duty. Whereas the alveolar membrane is largely composed of thin squamous cells, the intestinal mucosa is composed not only of mucin-covered tall columnar epithelium but also by a substantial tunica propria mucosae. The GI mucosa constitutes, therefore, a barrier against the intrusion of foreign materials that is many times more formidable than the alveolar membrane.

Penetration of the alveolar membrane, as an accompaniment that is best demonstrated by the presence of enlarged, discolored, and often stony-hard satellite lymph nodes. No such involvement of mesenteric lymph nodes has been described in coal miners or siliceous hard-rock miners even though they had ingested during their lifetime many times the amount of dust than finally remained stored in their lungs and satellite nodes. Consideration should be given to the fact that the dust stored in the lungs is estimated to represent only 1% to 2% of the dust that had been inhaled and, after clearance from the lungs, swallowed.

If penetration of finely divided foreign particles through the GI mucosa were a common event or even a relatively uncommon event, over the lifetime of an individual, the submucosa of the GI tract and the mesenteric lymph nodes would be the repositories for a considerable accumulation of a wide variety of materials other than those cleared from the lungs. One need only consider the wide variety of insoluble materials, mostly cellulosic, that are part of many foods. Some, like pepper, can be very irritating when in contact with unprotected tissue and would cause a severe inflammatory reaction. Furthermore, if penetration of the GI mucosa by particulates did occur, the amount of stored material in the intestinal submucosa and in mesenteric lymph nodes would increase with age. The fact, however, is that storage of such foreign material has not been seen in either the intestinal wall or the mesenteric nodes.

The failure to find fibers by electron microscopic examination in sections of intestinal wall of rats fed asbestos or taconite tailings is consonant with the failure to find evidence of transmigration in the digests of other tissues of these animals.

SUMMARY

The more important conclusions can be briefly summarized as follows:

1. Twenty-one months of intimate contact of rat GI mucosa with very high concentrations of chrysotile asbestos failed to produce cancer or any other kind of lesion. These results are highly important inasmuch as in earlier work different modes of asbestos exposure have resulted in cancer production within 16 to 17 months.

2. Twenty-one months of high-dosage-chrysotile feeding of rats resulted in no evidence of penetration of GI mucosa by the mineral fibers and no incontrovertible proof of transmigration as judged from electron microscopic studies.

3. Administration of a single dose of 400 mg of either amosite or taconite tailings by gavage provided no evidence of transmigration of fibers even though the method of examination used was many thousand times more sensitive than the method of examining tissue sections.

4. The voluntary intake by rats within six days of 2 to 10 gm of amosite or taconite tailings mixed in oleomargarine likewise provided no evidence of transmigration of mineral fibers, although a total of nine fibers was found in all of five rats that could have been considered evidence of transmigration, except for the possibility that they were contaminants.

5. Short-term and long-term feeding (up to 1½ years) of chrysotile or crocidolite by a second laboratory resulted in no tumor production in the GI tract or mesothelium during the lifetime of the animals. Light microscopic studies provided no evidence that penetration of tissues by ingested asbestos fibers occurred.

6. A partially completed light microscopic and electron microscopic study in a third laboratory has provided data that support the results and conclusions of the other two laboratories with regard to the failure of ingested asbestos fibers to penetrate tissues.

7. The penetration of the pulmonary alveolar membrane and transmigration of inhaled dust particles are facts readily demonstrable by the presence of enlarged, discolored, mineralized tracheobronchial lymph nodes. In contrast to this, the GI mucosa, constituting a much more formi-

ST0065522

dable barrier, does not permit the penetration of insoluble particulates as is evidenced by the lack of similar involvement of mesenteric lymph nodes in miners who, during their lifetimes, ingest nearly 100 times the amount of dust stored in their lungs and satellite lymph nodes, plus the considerable amount of insoluble particulates in the daily food intake. Neither is evidence of penetration or storage of inhaled or ingested foreign material to be found in the intestinal wall of these miners.

References

1. Gross P, et al: Mineral fiber content of human lungs: A comparison of the counts obtained from the lungs of people of Pittsburgh, Pennsylvania, with those from the lungs of people of Charleston, South Carolina. *Am Ind Hyg Assoc J*, to be published.

2. Langer AM: Discussion in Langer AM, et al: Inorganic fibers, including chrysotile, in lungs of autopsy: Preliminary report, in Walton WH (ed): *Inhaled Particles: III* Unwin Brothers, Limited, The Gresham Press, Old Woking, Surrey, England, 1971, vol 2, p 693

3. Policard A, quoted by Gordonoff T: Epuration pulmonaire, in *Compte Rendus des Journees Françaises de Pathologie* Minère, Paris, Charbonage de France, Oct 27-28, 1960, p 165.

4. Bonser GM, Clayson DB. Feeding of blue asbestos to rats, in the *25th Annual Reports of the British Empire Cancer Campaign*. 1967, p 242.

5. Smith WE: Asbestos, talc and nitrites in relation to gastric cancer. *Am Ind Hyg Assoc J* 34:227-228, 1973

6. Gross P, et al. Experimental asbestosis: The development of lung cancer in rats with pulmonary deposits of chrysotile asbestos dust. *Arch Environ Health* 15:343-355, 1967.

7. Gross P, Harley RA Jr: Asbestos-induced intrathoracic tissue reactions. *Arch Pathol* 96:245-250, 1973.

8. Pontefract RD, Cunningham HM: Penetration of asbestos through the digestive tract of rats. *Nature* 243:[illegible]-353, 1973

9. Gross P: Is short-fibered asbestos dust a biological hazard? *Arch Environ Health* 29:115-117, 1974.

10. Drinker CK, Field ME: *Lymphatics, Lymph and Tissue Fluid*. Baltimore, Williams & Wilkins Co, 1933.

11. Hamburger M, Robertson OH, unpublished experiments quoted by Robertson OH: Phagocytosis of foreign material in the lung. *Physiol Rev* 21:112, 1941.

ST0065523

MYTHOLOGY

Index of Gods, Etc

The Triple Gods

The triple gods [illegible]
And other [illegible]—
Sir William [illegible]
Authority for this recension—

The triple gods, I meant to say,
Were Bachus, Vulcan, Venus
Sir William's counsel is hygienous
And surely non-sclerotic though it may
Be justa nute restrictive, I remark
The lethal one of these is work.

Charon

I sing of Charon and his ferry—
The price is right,
Short is the flight,
But the journey is dismal—very.

—*Rusticatus*

(144)
cc- E & Blair
S. Spangler
J. Leddy
D. Sheets
C. Lichy
cc. GF 12/11/74

ST0065524

Ingested Mineral Fibers

Do They Penetrate Tissue or Cause Cancer?

Paul Gross, MD; Russell A. Harley, MD; Layinka Margaret Swinburne, MRCP, MRC Path; John M. G. Davis, PhD; William B. Greene

Three different laboratories independently investigated the ability of ingested mineral fibers to penetrate tissues in rats. All concluded that there was no evidence of tissue penetration by ingested mineral fibers.

These experimental observations are supported by the findings in coal and hard-rock miners who swallow, during their lifetime, nearly 100 times the amount of dust that is stored in their lungs. The intestinal wall or mesenteric lymph nodes of these people show no evidence of storage of the ingested dust particles. Animals fed asbestos over much of their lifetime and allowed to live to the age of cancer production, failed to provide evidence of a cancerogenic effect.

Submitted for publication May 31, 1974; accepted Aug 9, 1974.

From the Medical University of South Carolina, Charleston (Drs. Gross, Harley, and Mr. Greene), St. James's Hospital, Leeds, England (Dr. Swinburne), and the Institute of Occupational Medicine, Edinburgh (Dr. Davis).

Reprint requests to the Department of Pathology, Medical University of South Carolina, 80 Barre St, Charleston, SC 29401 (Dr. Gross).

Because of the association of peritoneal mesotheliomas with asbestos exposure, it was suggested that inhaled asbestos fibers cleared from the lungs and swallowed, migrated through the gastrointestinal (GI) wall to the peritoneum there to initiate the cancer. The more recent discovery of submicronic mineral fibers in beers, wines, and drinking water has resulted in publicized expressions of fear that a health hazard exists.

A recent investigation[1] suggests that the lungs of city dwellers not occupationally exposed to asbestos may contain many millions of submicron-sized fibers. A percentage of these fibers have been identified as chrysotile. Amphibole asbestos fibers have also been identified in the lungs of city dwellers but their amount or concentration has not been determined.[2]

Since only about 1% to 2% of the dust inhaled during a lifetime is retained in the lungs, the other 98% to 99% being transported via the pulmonary clearance mechanism to the pharynx,[3] it is probable that the GI tract will be the recipient of much of the inhaled and subsequently ingested dust particles, nonfibrous as well as fibrous.

Ingestion of asbestos fibers has undoubtedly been in progress in some countries for centuries, inasmuch as fibers of submicronic dimensions occur naturally in river water as it erodes serpentine and amphibole outcroppings. Increased industrial applications of mineral fibers may have accelerated this ingestion somewhat. Nevertheless, the world literature does not suggest that an alarming increase in GI inflammatory disease or cancer has occurred, or that the incidence is higher in regions where asbestos fibers are present in natural waters.

Because of the publicly expressed fears that ingestion of beverages or food containing submicronic mineral fibers may expose large populations to a cancer-producing hazard, responsible public officials are demanding that the scientific community confirm or refute the assertion that a health hazard exists. The present report is intended to be a response to this demand. A portion of the work here reported was begun nine years ago, long before the possible consequences of asbestos ingestion became of widespread interest. However, because of their negative nature, the results were not published. Two other laboratories more recently did publish negative results of asbestos feeding studies: one, as an abstract or short note[4]; and the other, as part of a discussion on the dangers of nitrites in food.[5]

When it was learned recently that three laboratories were either engaged in a study on asbestos ingestion or had completed it, agreement was reached to pool the results of these studies in the present joint paper.

Taconite tailings from an iron mine, containing submicronic mineral fibers have been piped into Lake Superior for nearly 20 years. Because some ultramicroscopic mineral fibers have been discovered recently in the drinking water from this lake, dust from this mineral is included in these studies.

MATERIALS AND METHODS

Chrysotile Feeding

Ground laboratory chow was intimately mixed with ball-milled chrysotile asbestos (consisting of the whole spectrum of fiber lengths and diameters) so that the latter constituted 5% by weight of the mix. This was supplied along with water ad libitum to ten male rats for 21 months at which time all were killed. There was no time period between the asbestos feeding and death to allow the GI tract to rid itself of the ingested asbestos. Five laboratory control rats of the same age as the asbestos-fed animals were killed at the same time as the latter.

The GI tract of the animals was carefully examined for lesions and random blocks of the tissue were removed for paraffin impregnation and subsequent sectioning. Blocks of tissue were also removed from the thoracic and other abdominal organs. The remainder of these tissues was placed together in a jar of formaldehyde solution for possible future study. Because no thought was given at that time to the possibility of transmigration of fibers, tissue from the GI tract was not isolated from other tissues and no attempt was made to keep the formaldehyde solution that had been contaminated by asbestos-containing fecal material from contaminating the other tissues with asbestos fibers.

Paraffin sections, cut at 6μm and stained with hematoxylin-eosin, were made of the various tissues. One-millimeter blocks were cut from the fixed tissue of the GI tract after nine years sojourn in formaldehyde solution and embedded in epoxy resin for electron microscopic examination. Weighed portions of the large and small intestines, and of the mesentery as well as of the other organs were processed as described below for determination of their mineral fiber content.

Administration of Fibrous Minerals by Gavage

Amosite and taconite mine tailings were finely ground in a mill (Spex) so that an aqueous suspension of either dust could be ejected through an 18-gauge needle. Unanesthetized rats were given 1 ml of suspension containing 400 mg of the dust by gavage, using an 18-gauge blunted needle. Because some of the animals struggled during the gavaging, a certain amount of trauma occurred. Ten rats were given amosite and ten, taconite. Five rats of the same age were left untreated as controls. Two gavaged rats from each series and one control animal were killed at 24 and 48 hours, and one, two, and three weeks later.

At autopsy, the GI tract was carefully removed so that the remaining tissues did not become contaminated with the content of the GI tract. Weighed portions of the various tissues were processed in a manner similar to that of the preceding study and according to the method to be described.

Oleomargarine Feeding

Because of the likelihood of traumatic hematogenous introduction of mineral fibers during the gavaging—a possibility recognized belatedly—a new series of animals was initiated. For this series, finely ground amosite and taconite tailings were each intimately incorporated into oleomargarine to obtain a 10% amosite content or a 20% taconite content. Rats were housed in individual cages (ten for each mineral) and deprived of all food except for 50 gm of the dust-containing oleo. During this time (six days), the dust consumption per rat ranged from 2.2 gm to 5 gm amosite and from 6.0 to 10 gm taconite. Ten control rats received no dust.

One, two, three, four, and five weeks after completion of the dust-feeding period, two rats from each series were killed. Two control rats were killed at the third, fourth, and fifth week, and one at the first week (three other control rats had died during the night). At autopsy, weighed portions of the GI tract and of other organs were processed according to the method described below. Blocks of intestinal wall fixed in formaldehyde, 1 mm in diameter, were removed for electron microscopic study.

Butter Feeding

In the second laboratory, three experiments were conducted: a preliminary short-term study, and two long-term experiments. The animals were SPF rats of the Wistar strain, initially about 12 weeks old. The asbestos was sieved to a fiber length up to 100μm and was incorporated in butter so as to result in either a 0.4% or 0.2% mixture.

1. In the preliminary study, a small number of rats were given a single dose of either 20 or 40 mg crocidolite asbestos. Fecal pellets were examined by optical microscopy and after monatomic ashing at 39.7 mm Hg, at 200 w, from between 15 minutes to one hour, at intervals from six hours to one week after feeding. The animals were killed from between 1 week to 19 days and were dissected so as to avoid contamination by gut contents. The gut and other tissues were fixed in formaldehyde solution. Paraffin sections (6μm thick) were examined by optical microscopy and samples of tissue were also subjected to monatomic ashing as described for fecal pellets.

2. Groups of rats were fed according to the following schedules once weekly for 16 weeks: 31 rats—10 mg Rhodesian chrysotile; 33 rats—5 mg crocidolite of unknown origin; and 34 rats—10 mg crocidolite of the same unknown origin. On the same schedule a control group of 24 rats was given the butter only. All rats were fed a normal diet of standard pelleted food in addition.

3. A group of 35 rats was fed once weekly for 18 weeks 10 mg of North West Cape crocidolite, and another group of 28 rats received 10 mg of Transvaal crocidolite on the same schedule. There was also a butter-fed control group of 24 rats. All of these rats were also additionally fed a normal diet of standard pelleted food.

A few animals from each group of each experiment were killed in the first six months to assess any possible intermediate pathological changes in the abdominal region or elsewhere. The remainder were allowed to survive until they became ill or died a natural death. At autopsy, any exudate present in the body cavities was aspirated and examined microscopically. Where no exudate was present, the cavities were washed out with a small volume of normal saline and also examined for the presence of cells or asbestos fibers. The lungs were inflated in situ with formaldehyde saline and removed intact.

All tissues suspected of neoplastic change were examined histologically. In addition, the major organs, abdominal wall, and chest wall, and lymph nodes were examined in a proportion of animals from each batch (53 in all). Samples of this tissue were also subjected to monatomic ashing to facilitate the recognition of asbestos residues.

Long-Term ad Libitum Feeding of Asbestos in Butter

These studies, in the third laboratory, on the penetration and transmigration of ingested asbestos fibers have not been com-

ST0065525

pleted but the data that have been obtained on a few animals are included here because they complement and supplement the information obtained by the other two laboratories.

Chrysotile and crocidolite, properly ground, were intimately mixed with butter to make two batches, each containing 5 mg/gm of butter of one or the other type of asbestos. Two groups of 12 rats each were fed one or the other type of asbestos ad libitum. Animals were killed one and three months after the start of ingestion and sections of the esophagus, stomach, small intestine, cecum, colon, mesenteric lymph nodes, liver, spleen, and lung were prepared for light microscopy and transmission electron microscopy.

Preparation of Tissue Digests (First Laboratory).—Weighed amounts of tissue generally between 1 and 5 gm were added to about 20 ml of 5% sodium hypochlorite (commercial bleach) and allowed to be digested. In order to accelerate the digesting process, the mixture was placed in an oven at 50 to 60 C. It often became necessary to add new hypochlorite solution to the tissue in order to carry the process to completion. When a clear solution was obtained, it was centrifuged and the sediment washed three times with large amounts of unfiltered tap water. Care was taken during the washing so as not to lose any sediment. The latter was finally made up to a volume of 2 ml with distilled water and 20μl of the well-mixed suspension was placed on an electron microscope grid. Photographs were taken of all structures that had a greater than 1:3 aspect ratio.

Differentiation of Electron Microscope-Size Fibers (First Laboratory).—Some preparations initially were not satisfactory and resulted in enigmas that were resolved when structures that had been interpreted as fibers by light microscopy were found to be needle-like crystals. These readily dissolved and disappeared when the suspension was acidified.

Digestion of Tissue vs Tissue Section: Relative Sensitivity of Methods.—Considering the weight of tissue in a section on an electron microscope grid to be about 1.5×10^{-3} mg and the amount of digested mesenteric tissue represented in a 20μl drop on an electron microscope grid to average about 28 mg, then the latter grid represents nearly 19,000 times as much tissue as the grid containing a section of tissue 1-mm square and 1μm thick. In other words, if only one fiber were found on a grid containing a digest of mesenteric tissue, it would have been necessary to examine approximately 19,000 sections in order to find that one fiber in the same tissue.

Although the digestion method is capable of sampling amounts of tissue many thousand times greater than can reasonably be sampled with the method using sections 1-mm square and 1μm thick, it also has the disadvantage of being susceptible to give false-positive results. These may be caused by incomplete digestion of tissues giving rise to fibers in approximately the same size range of those ingested, but distinguishable from the latter in other respects. Another, more troublesome, cause of false-positive results is contamination. The contamination could theoretically be avoided by careful technique during the autopsy of the animal, the removal and weighing of the tissues, and by conducting the digestion and the preparation of the digests as well as of the grids in a clean room supplied with filtered air. It would also be necessary to use only water and reagents that had been passed through a Millipore filter.

RESULTS

Chrysotile-Fed Rats (First Laboratory)

Although they had ingested a large amount of asbestos each day for almost two thirds of their lifetime, the rats had thrived and appeared healthy. There were no deaths in this group. At autopsy, no lesion could be found by gross examination in the GI tract of these animals. Random paraffin sections of the GI tract likewise revealed no important abnormality microscopically. Sections of the other organs showed no evidence of damage. As might have been expected, the digests of the tissues of the GI tract that included the mucosa and its covering material, when examined with the electron microscope, contained chrysotile fibers. However, no fibers were found in the sections of intestines examined with the electron microscope.

What appeared at first glance to be of considerable importance was the finding of fibers in the digests of mesenteric tissue of three rats. In one, a chrysotile fiber was found. In the other two, the fibers were nonchrysotile. In a fourth rat, two nonchrysotile fibers were identified in a digest of a mixture composed of cardiac, pulmonary, renal, and splenic tissues.

However, two control rats were also found to have chrysotile fibers in the tissue digest of the GI tract. One of these two controls also had a nonchrysotile fiber in the same digest. All fibers were, of course, submicronic in dimensions.

Asbestos and Taconite Dosage by Gavage (First Laboratory)

One rat, gavaged with amosite and killed 48 hours later was found to have a lacerated and perforated esophagus. Much of the amosite was extravasated into the paravertebral muscles in the form of a streak parallel to the esophagus. The digests of the mesentery, kidney, and lung of this animal contained fibers consistent with amosite as well as other fibers. This is the only rat out of eight gavaged with amosite and examined that showed fibers in the digest of tissues outside of the GI tract. (Two other rats gavaged with amosite had died overnight and were discarded.) In only one out of ten rats gavaged with taconite were fibers found in a digest of tissues other than those of the GI tract. In the digest of lung tissue from this animal, three fibers were found.

Six of eight rats given amosite showed fibers in the digest of GI tract tissues and similar findings were made in the digests of the GI tract tissues of two out of ten rats given taconite tailings. No digest of tissues from the GI tract of rats killed after the 48-hour period contained fibers. A chrysotile fiber was found in the digest of GI tissue of a rat gavaged with taconite tailings.

Of the five control rats killed with this series of gavaged animals, one was discarded because it died during the night. The digest of mesenteric tissue of one control contained two fibers. A fiber was also found in the digest of kidney from another control rat.

Feeding With Oleomargarine (First Laboratory)

The digests of the GI tract of all animals fed with margarine contained a few fibers that were consistent with those incorporated in the oleomargarine. A chrysotile fiber was found in the digest of a rat fed amosite.

A single fiber was found in the di-

ST0065526

gest of mesenteric tissue of one taconite tailings-fed rat. On the other hand, the digest of the mesentery of an amosite-fed rat contained a chrysotile fiber and in two other amosite-fed rats the digests of mesenteric tissue contained two fibers each. A fiber with the splayed end typical of asbestos was found in the digest of lung tissue from a rat fed amosite and killed two weeks later. The digests of the tissues of the other animals inclusive of those of the controls contained no fibers. Electron microscopic study of the sections of intestinal wall revealed no fibers. The findings so far enumerated are summarized in Table 1. It should be emphasized that all of the fibers found were of ultramicroscopic dimensions.

Butter Feeding (Second Laboratory)

Preliminary Experiment.—After a single dose of crocidolite, light microscopic examination showed abundant fibers in fecal pellets after 24 hours. Examination after monatomic ashing showed smaller numbers of fibers in 48-hour pellets; but thereafter none were detected by either method. Examination of the gut and other organs showed no fibers even after a 40 mg dose.

Long-term Experiments.—Study with the light microscope of the tissues of the animals as well as of the aspirates and washings of the serous cavities, revealed no evidence of transmigration of asbestos fibers. No mesothelioma was encountered in these animals. Although two breast cancers and one lymphoma of axillary nodes were noted in one asbestos-fed group, similar cancers were seen in the control group, three breast cancers, one lymphoma, and one sarcoma of the thigh (Table 2). No lesions of any kind were seen that could be related to the ingested asbestos.

Long-Term ad Libitum Feeding of Asbestos in Butter (Third Laboratory)

Light microscopic examination of all tissues of animals killed one and three months after the start of asbestos ingestion showed no asbestos fibers that had penetrated into the tissues. Electron microscopic examination of the small intestine and mesenteric lymph nodes is complete for the animals killed after one month, and far advanced for the rats killed after three months. No sign of penetration by asbestos fibers has been seen.

COMMENT

Chrysotile Feeding

Inasmuch as the rats in this study had been on the asbestos diet up to the time of death, the surface of the gut could be expected to be covered by a fiber-containing film of mucin. These fibers would and did become manifest in the digests of these tissues.

Although the presence of the fibers in the other tissues could conceivably be explained on the basis of transmigration, a more probable explanation would be that the surface of these tissues became contaminated with fibers while in the same jar with the cut segments of the GI tract.

The presence of fibers in the tissues of control animals can be explained only on the basis of several embarrassing circumstances. These are concerned with contamination: one, from the ambient air; another could have occurred when the technician was weighing the tissues. The weighing pan could have been covered by fiber-containing moisture derived from the GI tract of a previous weighing. A third source of contamination could have been the unfiltered water and reagents used in the preparation of the digests.

The following conclusions can safely be drawn from these results:

1. Twenty-one months of contact with high concentrations of chrysotile fibers caused no gross or microscopic lesion of the intestinal wall. Inasmuch as lung cancers[6] and other chest cancers[7] induced by asbestos in rats have made their appearance from 16 to 17 months after the first exposure, this 21-month exposure to extremely high dosage of asbestos dust should have been adequate for a cancerogenic action to become manifest if such a potential were present in this mode of asbestos exposure.

2. Electron microscopic examination of the gut wall disclosed no evidence of transmigration of fibers.

3. Although the method of examining the sediment of digested tissues for the presence of fibers allows for the scanning of the equivalent of a volume of tissue many thousand times greater than can be examined on an electron microscope grid, the former has not yielded incontrovertible proof that transmigration of fibers occurs. Only one chrysotile fiber was found in the digests of tissues other than those of the GI tract and this, in only one animal out of ten. Furthermore, the importance of this finding becomes doubtful in view of the fact that tissue digests of two control rats also were found to contain chrysotile fibers, and in the tissue digest of one of these controls, a nonchrysotile fiber was found.

Administration of Fibrous Minerals by Gavage

The widespread dissemination of amosite fibers in tissues outside of the GI tract of the rat in which the perforation of the esophagus and extravasation of amosite into paravertebral muscles occurred is reminiscent of the experiment of Pontefract and Cunningham.[8] These writers performed laparotomies on rats and injected asbestos dust through the gastric wall. Here, the needle tore through tissues, opening vessels in the path of the needle. Furthermore, the needle track, remaining open for a time, allowed leakage of fibers into the abdominal cavity and onto the cut edges of the laparotomy. When these workers concluded that asbestos fibers transmigrated from the GI tract to practically all tissues of the body inclusive of blood, they ignored the artifacts inherent in their technique.

It is surprising that, with the potentially traumatic technique of gavaging, only one of 18 rats gave evidence of the widespread dissemination of fibers characteristic of the Pontefract and Cunningham technique and that in another gavaged rat, three fibers were found in the digest of lung tissue.

In spite of the indication that false-positive evidence of transmigration could have been obtained (as indi-

ST0065527

cated by the presence of a chrysotile fiber in the digest of GI tissue of a rat given taconite tailings, and the presence of a fiber consistent with either amosite or taconite tailings in the digest of mesenteric tissue of a control rat), it is noteworthy that following the administration of a single large dose (400 mg) of either amosite or taconite, no evidence of transmigration of fibers was observed in 16 of the 18 rats gavaged.

Oleomargarine Feeding

The results of this portion of the investigation indicate that if transmigration of mineral fibers from the GI tract occurs at all, it must be a rare event since only five fibers consistent with those ingested were found in the digests of mesentery of three rats and four such fibers were found in the lung digests of two other rats (Table 1). Other fibers (five in all) found in the digests of mesenteric tissue of three rats probably represented contaminations—such as the chrysotile fiber in the digest of the rat fed amosite.

The importance of the nine fibers (Table 1) found in the tissue digests of five of the 37 rats fed or gavaged with mineral fibers must be evaluated in the perspective of the billions on billions of fibers that had been introduced into the GI tract of these animals. If transmigration of fibers through the GI wall were a fact, one would logically expect more than one to three fibers out of many billions to accomplish this feat, and this phenomenon should be demonstrable in more than five out of 37 rats. The dose of asbestos ingested by the rats is equivalent to a consumption of 1.1 to 2.5 kg asbestos by a 75-kg man in six days, and double this amount in terms of taconite tailings. The importance of the nine fibers found is reduced to a negligible level by the finding in four rats of five fibers that are obvious contaminants, thus pointing to a very high probability that the nine fibers in question are also contaminants. As indicated previously, all fibers were ultramicroscopic in dimensions, and could have been derived from the air of the laboratory, as well as from the unfiltered water and the technique used (weighing of the tissues) in the preparation of the study samples.

Butter Feeding

The failure to detect penetration of the GI mucosa or transmigration of optically visible fibers in this study is important since asbestos fibers in this size range are known to be pathogenic, and there is a growing body of evidence to indicate that asbestos fibers in the submicronic size range are not pathogenic.[9] The fact that no cancer of the GI tract or mesothelium developed after long-term exposure of the GI mucosa to large numbers of asbestos fibers is highly significant.

Long-Term ad Libitum Feeding of Asbestos in Butter

The lack of success in the third laboratory using optical as well as electron microscopy in finding evidence of penetration of asbestos fibers after one and three months of ingestion of these mineral fibers respectively, adds weight to the importance of these negative findings in the other two laboratories.

GENERAL COMMENTS

The method of digestion used in this investigation by one laboratory is faulted by the possibility of chance contamination that may be difficult to evaluate and thereby give rise to false-positive results. The possibility of false-negative results also exists. For instance, fibers may be lost by incomplete digestion of the tissue, by incomplete sedimentation during centrifugation, and by being covered with partially digested material on the electron microscope grid. However, in view of the ratio of the amount of tissue examined by the digestion method as compared with that examined by the electron microscope section method (many thousands to one), the chance of a negative result with the former when the latter is positive would appear to be negligible.

Although transmigration of large objects such as bullets from one portion of the body to another has been documented, this transmigration is concerned with foreign materials that had already achieved penetration into the body interstitium. The penetration of inhaled dust particles through the alveolar membrane and the subsequent lymphatic transport to satellite nodes has been recognized and studied experimentally for more than a century but the mechanism by which this penetration takes place is still unknown. Drinker and Field[10] did determine that particles may penetrate the alveolar membrane and appear in the lymphatic fluid within minutes after their insufflation into the lung of a dog. This rapid penetration of the alveolar membrane by

Table 1.—Tabulation of Fibers Found in Digests of Tissues*

Tissue Digest	Dust	Fibers Found: No.	Fibers Found: Week-Rat†	Contaminating Fibers (Chrysotile and Others): No.	Contaminating Fibers (Chrysotile and Others)
Mesentery	Amosite 7 rats	2	2 wk	1	1 wk (chrysotile)
		2	5 wk		
	Taconite tailings 10 rats	1	1 wk	1	2 wk (chrysotile) GI tract
	Control (no dust) 4 rats			2	
Lung or kidney	Amosite 10 rats	1	2 wk (lung)		
	Taconite tailings 10 rats	3	2/7 wk gavage (lung)		
	Control (no dust) 7 rats			1	(kidney)
Total	48 rats	9		5	

* Electron microscopic examination.
† Week-rat refers to the animal killed at specified time.

ST0065528

Table 2.—Tabulation of Tumors Found in Rats Fed Asbestos

Types of Tumor	Experiment 2				Experiment 3		
	Chrysotile, 10 mg	Crocidolite, 5 mg	Crocidolite, 10 mg	Control	NW Cape Crocidolite, 10 mg	Transvaal Crocidolite, 10 mg	Control
Mesothelioma	0	0	0	0	0	0	0
Carcinoma	2 (breast)	0	0	3 (breast)	0	0	0
Sarcoma	0	0	0	1 (thigh)	0	0	0
Lymphoma	0	0	1 (axillary nodes)	1	0	0	0
No. of animals	31	33	34	24	35	28	24

particles was confirmed by Hamburger and Robertson[11] using a different technique.

There are, however, important differences between the alveolar membrane that protects the lung interstitium from intrusion by foreign material and the GI mucosa that, among other functions, also performs a similar guard duty. Whereas the alveolar membrane is largely composed of thin squamous cells, the intestinal mucosa is composed not only of mucin-covered tall columnar epithelium but also by a substantial tunica propria mucosae. The GI mucosa constitutes, therefore, a barrier against the intrusion of foreign materials that is many times more formidable than the alveolar membrane.

Penetration of the alveolar membrane, as an accomplished fact, is best demonstrated by the presence of enlarged, discolored, and often stony-hard satellite lymph nodes. No such involvement of mesenteric lymph nodes has been described in coal miners or silicotic hard-rock miners even though they had ingested during their lifetime many times the amount of dust than finally remained stored in their lungs and satellite nodes. Consideration should be given to the fact that the dust stored in the lungs is estimated to represent only 1% to 2% of the dust that had been inhaled and, after clearance from the lungs, swallowed.

If penetration of finely divided foreign particles through the GI mucosa were a common event or even a relatively uncommon event, over the lifetime of an individual, the submucosa of the GI tract and the mesenteric lymph nodes would be the repositories for a considerable accumulation of a wide variety of materials other than those cleared from the lungs. One need only consider the wide variety of insoluble materials, mostly cellulosic, that are part of many foods. Some, like pepper, can be very irritating when in contact with unprotected tissue and would cause a severe inflammatory reaction. Furthermore, if penetration of the GI mucosa by particulates did occur, the amount of stored material in the intestinal submucosa and in mesenteric lymph nodes would increase with age. The fact, however, is that storage of such foreign material has not been seen in either the intestinal wall or the mesenteric nodes.

The failure to find fibers by electron microscopic examination in sections of intestinal wall of rats fed asbestos or taconite tailings is consonant with the failure to find evidence of transmigration in the digests of other tissues of these animals.

SUMMARY

The more important conclusions can be briefly summarized as follows:

1. Twenty-one months of intimate contact of rat GI mucosa with very high concentrations of chrysotile asbestos failed to produce cancer or any other kind of lesion. These results are highly important inasmuch as in earlier work different modes of asbestos exposure have resulted in cancer production within 16 to 17 months.

2. Twenty-one months of high-dosage-chrysotile feeding of rats resulted in no evidence of penetration of GI mucosa by the mineral fibers and no incontrovertible proof of transmigration as judged from electron microscopic studies.

3. Administration of a single dose of 400 mg of either amosite or taconite tailings by gavage provided no evidence of transmigration of fibers even though the method of examination used was many thousand times more sensitive than the method of examining tissue sections.

4. The voluntary intake by rats within six days of 2 to 10 gm of amosite or taconite tailings mixed in oleomargarine likewise provided no evidence of transmigration of mineral fibers, although a total of nine fibers was found in all of five rats that could have been considered evidence for transmigration, except for the probability that they were contaminants.

5. Short-term and long-term feeding (up to 1½ years) of chrysotile and crocidolite by a second laboratory resulted in no tumor production in the GI tract or mesothelium during the lifetime of the animals. Light microscopic studies provided no evidence that penetration of tissues by ingested asbestos fibers occurred.

6. A partially completed light microscopic and electron microscopic study in a third laboratory has provided data that support the results and conclusions of the other two laboratories with regard to the failure of ingested asbestos fibers to penetrate tissues.

7. The penetration of the pulmonary alveolar membrane and transmigration of inhaled dust particles are facts readily demonstrable by the presence of enlarged, discolored, mineralized tracheobronchial lymph nodes. In contrast to this, the GI mucosa, constituting a much more formi-

ST0065529

dable barrier, does not permit the penetration of insoluble particulates as is evidenced by the lack of similar involvement of mesenteric lymph nodes in miners who, during their lifetimes, ingest nearly 100 times the amount of dust stored in their lungs and satellite lymph nodes, plus the considerable amount of insoluble particulates in the daily food intake. Neither is evidence of penetration or storage of inhaled or ingested foreign material to be found in the intestinal wall of these miners.

References

1. Gross P, et al: Mineral fiber content of human lungs: A comparison of the counts obtained from the lungs of people of Pittsburgh, Pennsylvania, with those from the lungs of people of Charleston, South Carolina. *Am Ind Hyg Assoc J*, to be published.
2. Langer AM: Discussion in Langer AM, et al: Inorganic fibers, including chrysotile, in lungs of autopsy: Preliminary report. in Walton WH (ed): *Inhaled Particles: III*. Unwin Brothers, Limited, The Gresham Press, Old Woking, Surrey, England, 1971, vol 2, p 693.
3. Policard A, quoted by Gordonoff T: Epuration pulmonaire, in *Compte Rendus des Journées Françaises de Pathologie* Minére, Paris, Charbonage de France, Oct 27-28, 1960, p 165.
4. Bonser GM, Clayson DB: Feeding of blue asbestos to rats, in the *45th Annual Reports of the British Empire Cancer Campaign*. 1967, p 242.
5. Smith WE: Asbestos, talc and nitrites in relation to gastric cancer. *Am Ind Hyg Assoc J* 34:227-228, 1973.
6. Gross P, et al: Experimental asbestosis: The development of lung cancer in rats with pulmonary deposits of chrysotile asbestos dust. *Arch Environ Health* 15:343-355, 1967.
7. Gross P, Harley RA Jr: Asbestos-induced intrathoracic tissue reactions. *Arch Pathol* 96:245-250, 1973.
8. Pontefract RD, Cunningham HM: Penetration of asbestos through the digestive tract of rats. *Nature* 243:352-353, 1973.
9. Gross P: Is short-fibered asbestos dust a biological hazard? *Arch Environ Health* 29:115-117, 1974.
10. Drinker CK, Field ME: *Lymphatics, Lymph and Tissue Fluid*. Baltimore, Williams & Wilkins Co, 1933.
11. Hamburger M, Robertson OH, unpublished experiments quoted by Robertson OH: Phagocytosis of foreign material in the lung. *Physiol Rev* 21:112, 1941.

MYTHOLOGY

Index of Gods, Etc

The Triple Gods

The triple gods of hypertension
And other osclerosis—
Sir William Osler is
Authority for this recension—

The triple gods, I meant to say,
Were Bachus, Vulcan, Venus.
Sir William's counsel is hygienous
And surely non-sclerotic, though it may
Be justa nute restrictive; I remark
The lethal one of these is work.

Charon

I sing of Charon and his ferry—
The price is right,
Short is the flight,
But the journey is dismal—very.

—Rusticatus

ST0065530