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Asbestos Contamination of Parenteral Drugs

Abstract. *Chrysotile asbestos* has been found in approximately one third of the samples from two sets of 17 widely used parenteral drugs.

Appropriate filtration procedures for sterilization and removal of foreign matter from parenteral solutions became necessary several decades ago (1). The pharmaceutical industry found asbestos filters useful and effective for this purpose, and they have become widely used.

Unfortunately, we have found that such filters add a contamination of their own to the filtrate and that, at least in the past 3 years, parenteral solutions used for intravenous, intramuscular, and intraperitoneal therapy in the United States have often contained measurable amounts of asbestos.

The physicochemical properties of asbestos make it highly suitable for filtration, and it is therefore widely used in chemical, food, drug, and other industries. The individual fibers and fibrils are both fine and strong, ranging in diameter from 200 Å to tenths of micrometers and in tensile strength from 20,000 to 60,000 kg/cm².

Hydrated magnesium silicate minerals, they tend to be chemically inert and resist attack by most chemicals (2). There are several varieties of asbestos (chrysotile, amosite, crocidolite, anthophyllite, tremolite), and special properties of each can be utilized for specific filtration purposes. Thus, crocidolite has been used for gas mask and cigarette filters. Chrysotile is widely used in food processing industries (sugar, lard, beer, and so forth). For pharmaceutical use, chrysotile again has been the asbestos variety of choice.

These filters are used at many points in the manufacturing process, from the preparation of raw materials, through the intermediate steps, to the final filtration of the product. The latter is important: in order to minimize or prevent bacterial growth (and the presence of bacterial toxins), it is often desirable to complete production procedures within 8 hours or less. Speed of final filtration may be critical in such production schedules. Chrysotile allows rapid filtration without reduction of efficacy.

Fig 1. (a) Chrysotile asbestos observed by polarized light microscopy from a single-vial sample of tetracycline. This particular fiber was 1 mm in total length (magnification $\times 1300$). (b and c) Chrysotile observed by electron microscopy in ampicillin and vancomycin, respectively (magnification $\times 25,000$).

Another reason for chrysotile's effectiveness as a filter is the possibility of its fragmentation in solution. The fibers are not unit structures, but rather bundles of ultramicroscopic fibrils, each 200 to 400 Å in diameter and 1000 Å to several micrometers in length (3). Untreated, the fibers in a filter can fragment into a much larger number of fibrils, the number of effective filtering elements being vastly increased and their surface area concomitantly extended.

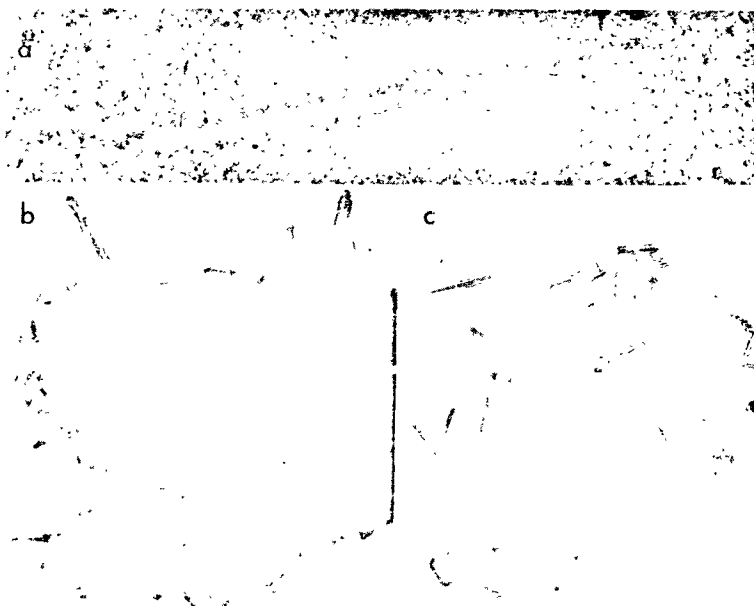
Serious health hazards have been identified with asbestos exposure (4); these include pulmonary fibrosis and a variety of neoplasms (such as bronchogenic carcinoma, pleural and peritoneal mesothelioma, and perhaps other neoplasms). Once thought to be limited to heavily exposed asbestos workers, these neoplasms are now known to occur with much less exposure, such as that experienced by those living in the household of an asbestos worker (5), working in shipyards in which asbestos is used in some areas (6), or merely living within 800 m of an asbestos plant (5, 7) or in the vicinity of asbestos operations (8).

Such human disease has been the result of inhalation of asbestos fibers. It

is not now known whether it occurs as the result of other routes of administration, although ingestion may be suspect in view of some evidence of increased incidence of gastrointestinal cancer among asbestos workers (9). In the experimental animal, it has been demonstrated that parenteral administration (typically, of 10 to 30 mg) of all varieties of asbestos can produce neoplasms (10). Such neoplasms have appeared at the site of injection of fibers. Other studies have also shown that administered fibers are readily disseminated from the injection site, both hematogenously and along draining lymphatic channels (11).

An investigation of a number of parenteral drugs has been undertaken. Single-dose vials of widely used drugs for parenteral injection, taken from the pharmacy stock of Mount Sinai Hospital during 1969 and again about 1 year later, were examined for their asbestos content. Analysis for chrysotile asbestos was made by using optical and electron microscopy.

Samples for analysis were obtained by filtering the contents of each vial through multipore AA membrane filters (diameter 13 mm, pore size 0.8 μ m). Drugs received from manufacturers as powders were prepared with sterile water, which in turn had been filtered through membrane filters (pore size 0.8 μ m); drugs received as solutions



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were filtered with no additional dilution. While the effective pore size of the membrane filters used is larger than the diameter of many of the asbestos fibers of interest, it was found that the surface-charge properties of the filters and the asbestos fibers, as well as the circuitous path through a filter, allow virtually complete collection of all asbestos material.

At the time of preparation of the drug samples, control samples were prepared with filtered sterile water. One such control was processed with each of four drug samples. All were prepared in a laminar-flow, filtered air hood, and strict clean-room procedures were followed as normal room air may provide a ready source of contamination.

Samples were prepared for optical microscopic examination by mounting one quarter of the membrane filter with collected material on a microscope slide. The filter was cleared for viewing with a 10 percent solution of membrane filter material in a 1:1 mixture of diethyl oxalate and dimethyl phthalate. The cleared filter was scanned by means

of polarized light microscopy at a magnification of $\times 400$.

All birefringent fibers longer than $5 \mu\text{m}$ and with a length-to-width ratio greater than 5 were counted. Whenever possible, identification of chrysotile asbestos was made on the basis of extinction angle and morphology. This identification could be made with reasonable assurance only for those fibers longer than about $100 \mu\text{m}$.

For identification of asbestos by electron microscopy, one half of the filter and collected material was ashed in a low-temperature activated oxygen asher (12). The residue was dispersed by grinding for 2 to 5 minutes in a 1 percent solution of nitrocellulose dissolved in amyl acetate, and allowed to dry. A known fraction of the dried film was transferred to a 200-mesh, Formvar-coated electron microscope grid. The prepared grids were then scanned at a magnification of $\times 42,000$, and the number of asbestos fibrils and their size were determined in two to four grid squares (100 by $100 \mu\text{m}$). In some selected cases, the residue from a par-

tially ashed membrane filter along with collected residue was mounted directly on the electron microscope grid. In all cases, the identification of chrysotile asbestos was made on the basis of its unique morphology (3). Other tubular minerals, such as halloysite, exist in nature, but their presence in these samples can be ruled out on the basis of size, geologic rarity, and absence from manufacturing processes for parenteral drugs.

During 1969, 16 samples were scanned optically, and all fibers longer than $5 \mu\text{m}$ were recorded. Fibers longer than $100 \mu\text{m}$ that could be identified with reasonable certainty as asbestos were noted. The results of this analysis are given in Table 1 (13). Many fibers and particles, in addition to asbestos, were present in the filtered samples. They included cellulose fibers, starch granules, and inorganic and organic fibers of unidentified composition. Moreover, in optical scanning, numerous fibers were seen that were consistent with asbestos, but identification could not be made reliably and they were

not recorded. This portion of the sample was later found by

To confirm the results of the 16 samples, a duplicate of each sample was scanned by electron microscopy. In five of the samples, including all the fibers were identified as asbestos. Examples of asbestos in background samples by mean electron microscopy are shown in Figures 1b and 1c.

During fall 1970, a second set of drug samples was prepared to ascertain if asbestos might still be found in parenteral drug preparations. Only one sample was undertaken. The results are given in Table 1. The results indicate that parenteral drugs by common occurrence of asbestos in 6 of the samples, and the amount of asbestos in 6 of the samples exceeded background levels.

The amounts of asbestos in these samples are reported for comparison with those reported for other parenteral drugs. Asbestos is measured in ambient air, typically in the range of 10 to 100 fibers per cubic meter. Concentrations of 100 nanograms or micrograms per liter may be evaluated with a filter that 10^{-9} g of asbestos fibers of a size of 0.5 μm in diameter (400 A in diameter). It should be noted that these results do not guarantee the absence of asbestos in the drug sample. The amount of asbestos in the sample was taken as an inadequate sample for analysis. Moreover, the results represent only a small portion of the total asbestos in the market. On the basis of the findings of asbestos in the single vials that were analyzed, this investigation overstates the significance of the contamination of some parenteral drugs.

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Table 1. Fibrous particulates seen in samples of parenteral drugs obtained during two periods of time. Observations were made by optical microscope (O) or electron microscope (E). The optical determinations were made on one quarter of each sample, the electron-microscope determinations on 10^{-7} of each sample. All drugs were labeled for intravenous use except those identified by IM for intramuscular or IP for intraperitoneal. Amounts of samples are in grams unless specified otherwise. "Yes" after optical data indicates that some fibers in the sample were identified as chrysotile by optical microscopy; while many fibers seen in all samples could have been asbestos, the majority were not. All values of 27×10^{-9} g or less for the estimated mass of asbestos could have resulted from the analysis background; N, number.

Drug	Amount (g)	Fibers by O, 1969			Asbestos	Chrysotile fibrils by E		Asbestos estimated mass in total sample (10^{-9} g)
		5-20 μm (N)	20-100 μm (N)	> 100 μm (N)		1969 (N)	1970-71 (N)	
Sodium acetazolamide	1	52	35	7			> 100	1100
Sodium ampicillin	1	10	13	3	Yes	32	2	76
Cephaloridine	2	38	28	4			85	210
Sodium cephalothin	2	44	22	5		8	6	7.7
Chloramphenicol	2						8	14
Cortisone (IM)	0.25	6	2	1			2	4.3
Lincomycin HCl	1.2	2	4	0			4	10
Sodium methicillin	2	91	51	10		6	22	82
Neomycin sulfate* (IM)	1	28	6	1			2	17
Neomycin sulfate* (IP)	5	101	57	38	Yes	79	1	39
Sodium oxacillin	2	24	35	18			> 100	410
Streptomycin sulfate* (IM)	1	15	0	0			4	7.5
Streptomycin sulfate* (IM)	2	15	8	1				
Sulfisoxazole diolamine	4	24	7	1			8	15
Tetracycline HCl†	1	26	16	7	Yes	50	35	86
Tetracycline HCl†	0.5					15		
Vancomycin HCl	1	16	7	1		26	30	78
Potassium penicillin G	10^6 units	14	4	1			12	14
Sodium penicillin G	2×10^6 units						9	27
Water control	20 cm^3	6	0	0		3	7	101

* Duplicate analyses of samples from different manufacturers of the same drug. † Duplicate analyses of samples from the same lot of a manufacturer. ‡ Average of seven samples ranging from 1.2×10^{-9} to 2.1×10^{-9} g.

brane filter along with was mounted directly on microscope grid. In all detection of chrysotile on the basis of its (3). Other tubular as halloysite, exist in presence in these ruled out on the basis of rarity, and absence of processes for par-

16 samples were, and all fibers longer recorded. Fibers longer that could be identified with certainty as asbestos. Results of this analysis (13). Many fibers in addition to asbestos, the filtered samples cellulose fibers, starch organic and organic identified composition. scanning, numerous that were consistent identification could reliably and they were

were made by optical microscopy-microscope determination of IP for some fibers in the samples, the majority were not N, number.

Asbestos, estimated mass in total sample (10 ⁻⁶ g)	
1970-71 (N)	1970-71
>100	1100
2	7.6
85	210
6	7.7
8	14
2	4.3
4	10
22	82
2	1.7
1	3.9
>100	410
4	7.5
8	15
35	86
30	78
12	14
9	27
7	101

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not recorded as asbestos. Figure 1a shows a portion of a chrysotile asbestos fiber found by optical microscopy.

To confirm the presence of asbestos, 6 of the 16 samples taken in 1969 and a duplicate of one of the positive samples were scanned by electron microscopy. In five of the seven cases, including all those in which asbestos fibers were identified optically, concentrations of asbestos several times those in background controls were found. Examples of asbestos found in two samples by means of transmission electron microscopy are shown in Fig. 1, b and c.

During fall 1970 and spring 1971, a second set of drug samples was investigated to ascertain whether asbestos might still be found in typical parenteral drug preparations. In this second study, only ultramicroscopic analysis was undertaken of the residue from filtered drugs. The results shown in Table 1 indicate that contamination of parenteral drugs by asbestos was still a common occurrence. Here, the quality of asbestos in 6 of 17 samples significantly exceeded background levels.

The amounts of asbestos estimated in some of these samples exceeded 2 microgram and were much higher than those reported for other environmental circumstances. Asbestos concentrations measured in ambient air, for example, are typically in the range of nanograms per cubic meter. Contamination with nanograms or micrograms of asbestos may be evaluated with the knowledge that 10⁻⁸ g of asbestos might represent 10⁶ fibrils of a size typically seen in drugs (400 Å in diameter by 1000 Å in length). It should be noted that negative or indefinite results for a particular sample do not guarantee the absence of asbestos in the drug lot from which the sample was taken. A single vial is an inadequate sample of a large production run. Moreover, the drugs sampled represent only a small fraction of those on the market. On the other hand, the finding of asbestos in one third of the single vials that were examined in this investigation over a 1-year period indicates significant asbestos contamination of some parenteral drugs at this time.

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Crustacean Color-Change Hormone:

Amino Acid Sequence and Chemical Synthesis

Abstract. The blanching hormone of the prawn, *Pandalus borealis*, is pGlu-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH₂. Its structure was settled by a combination of mass spectrometry and Edman-dansyl analysis of a thermolysin fragment. Confirmation of the structure was obtained by chemical synthesis from amino acids. This neurosecreted hormone is active in picogram amounts when tested in shrimps.

Many crustaceans have the ability to change their body color in order to match their background. These color changes are brought about by hypodermal chromatophores, that is, specialized cells containing movable pigment granules and having richly ramified cell processes. Crustacean chromatophores are controlled via neurosecreted hormones, which are released from nerve endings in the sinus gland, a neurohemal organ located in the eyestalks of most decapod crustaceans (1).

One color-change hormone, the blanching (red-pigment-concentrating) hormone, has been isolated from eyestalks of the prawn, *Pandalus borealis* (2). This hormone, which is active in shrimps in picogram amounts (3), is present in very minute amounts in the crustacean eyestalks (3) and, therefore, only about 90 µg of the pure blanching hormone has been available for our studies of its structure. The hormone was found to be a small peptide with a blocked NH₂-terminus (2), and we now report the deduction of its complete structure, which we have confirmed by chemical synthesis.

Quantitative amino acid analysis (4) on 8 µg of the isolated hormone (after hydrolysis in 6M HCl at 110°C for 24 hours at reduced pressure) and determination of its tryptophan content by ultraviolet spectroscopy (2) gave the following composition: Asp_{1.23}-

Glu_{0.99} Gly_{0.98} Leu_{1.14} Phe_{0.86} Pro_{1.01} Ser_{1.18} Trp_{1.00} (5), which accounts for 99 percent of the weight of the hormone.

The hormone is electrophoretically immobile at acid, neutral, and alkaline pH (6), in accordance with its blocked NH₂-terminus, and proving that none of its carboxyl groups is free.

Digestion of the hormone (23 µg) with thermolysin and fractionation of the digest on a column of Sephadex G-25 gave two major peptide fragments. Upon acid hydrolysis, one of these gave aspartic acid, glutamic acid, and leucine in about equimolar proportions. Since no free NH₂-terminal group was obtained by the DNS-Cl method (7), this peptide was an NH₂-terminal fragment of the hormone. Acid hydrolysis of the other peptide yielded about equimolar amounts of glycine, phenylalanine, proline, and serine. In addition, it contained tryptophan, which was determined by ultraviolet spectroscopy. It had a free NH₂-terminus, and analysis by the Edman-dansyl method, essentially as described by Gray and Smith (8), established its sequence as Phe-Ser-Pro-Gly-Trp-NH₂. The COOH-terminus was identified by omitting the hydrolysis after the DNS-Cl treatment which followed a four-cycle Edman degradation. The DNS product so obtained was identical to a reference DNS-tryptophan amide when compared in polyamide thin-layer chromatography