DISSOLUTION OF GLASS FIBERS IN THE RAT LUNG FOLLOWING INTRATRACHEAL INSTILLATION

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ABSTRACT

The biopersistence of airborne fibers is felt to play an important role in their potential toxicity. Since the dissolution rate of fibers can be measured in cell-free systems, the current study was undertaken to determine if the dissolution rate of fibers in the lung was related to the dissolution rate of fibers in vitro, and whether dissolution serves to remove fibers from the lung. To determine dissolution rates in vivo, suspensions of fibers were administered to rats by intratracheal instillation, and the numbers, lengths, and diameters of fibers recovered from the lungs at intervals up to 1 yr after administration were measured by phase-contrast optical microscopy. Five different glass fibers were used that had dissolution rates ranging from 2 to 600 ng/cm^2/h measured in vitro in simulated lung fluid at pH 7.4. Examination of the diameter distributions of fibers longer than 20 μm showed that the peak diameter decreased steadily with time after instillation, at the same rate measured for each fiber in vitro, until it approached zero. Measurements of the total number of fibers remaining in the rats' lungs at times up to 1 yr after instillation suggest that not many of the administered fibers were being cleared by macrophage-mediated transport via the conducting airways. A computer simulation of the fibers in the lungs was performed in which each of the administered long fibers (20 μm or longer) was decreased in diameter according to the rate measured in vitro, while the short fibers (less than 20 μm long) were unaffected. The ratio of long to short fibers predicted by this simulation agreed well with this quantity measured from the fibers recovered from the rats' lungs at each time interval after instillation. It was concluded that long glass fibers, at least those longer than 20 μm, are removed from the lung by dissolution at much the same rate measured in vitro.

INTRODUCTION

The potential biological activity of airborne fibers has long been linked to the dose, dimension, and durability of the fibers (Stanton & Wrench, 1972). Extensive studies of the concentrations (potential doses) of airborne fibers are available, as well as data on the size (dimensions) of both airborne fibers and those fibers found in human and experimental animal lungs. Recently, interest in the role of the biopersistence of fibers (durability) has increased, as evidenced by a recent conference on biopersistence, supported by the International Agency for Research on Cancer (IARC, 1994).

Measurements of the dissolution rate of fibers in cell-free systems in the laboratory have been reported by several investigators (Leineweber, 1984; Scholze & Conradt, 1987). In a similar manner, a number of reports of fiber alteration following residence in rodent or human lungs have been made (Bellmann et al., 1987; Morgan et al., 1982; Morgan, 1994). Recently, we (Potter & Mattson, 1991; Mattson, 1994) have reported methods for assessing the dissolution rate of fibers in vitro and the factors needed to make the results consistent and relevant to dissolution in vivo.

The ability to measure accurately the dissolution rate of fibers in cell-free systems is useful, but unless the behavior of fibers in the lung is somewhat similar or predictable based on these measures, the utility of laboratory measurements is limited.

The study reported here was carried out to determine whether the dissolution rates of glass fibers measured in simulated lung fluid at neutral pH resembled those in the rat lung. Glass fibers of five different compositions were followed after intratracheal instillation in rats. Three of these, with dissolution rates of 2, 150, and 600 ng/cm^2/h, were produced by drawing a continuous fiber from the melt through a one-hole bushing and chopping it. These fibers had a fairly consistent diameter near 2 μm, but varying lengths from below 5 to 100 μm or more. Two other fiber compositions with dissolution rates of 100 and 300 ng/cm^2/h were samples of the 2 glass fibers that were recently tested in chronic inhalation studies at the Research and Consulting Company in Geneva, Switzerland (Hesterberg et al., 1993).

METHODS

The compositions for the five fiber types used in this study and their dissolution rate constants measured in vitro are shown in Table 1. The rate constants, which ranged from 2 to 600 ng/cm^2/h, were measured in simulated lung fluid at pH 7.4 at a flow rate high enough that the result was independent of the flow rate (Mattson, 1994). The dissolution rate of glass fibers is a sensitive function of the composition, which accounts for the substantially different dissolution rate constants of the fibers chosen (Potter & Mattson, 1991). Measurements of the mean length and diameters of the fiber samples, and some associated statistical parameters, are given in Table 2.

The three fibers, identified as X7779, X7753, and X7484, were produced as continuous filaments by melting essentially pure chemicals and drawing the glass through a one-hole bushing. Bundles of these filaments were prepared and embedded in an epoxy resin (JB4, Polysciences, Inc., Warrington, PA). Slices approximately 50 μm thick were cut from the blocks with a rotary end mill, and, after removing the resin by low temperature ashing, the fibers were collected and mixed to ensure homogeneity. The average lengths of these fibers were considerably shorter than the 50 μm slice milled from the block, probably because the fibers broke during the milling process. Their compositions were calculated from the amounts of materials melted.

TABLE 1. Compositions of the Fibers Used in the Study in Oxide Weight Percent and Some of Their Properties

Composition and properties	X7779	X7753	X7484	MMVF10	MMVF11
SiO_2	60.1	65.7	60.7	57.4	63.5
Al_2O_3	8.1	0.5	3.8	5.2	3.8
B_2O_3	1.8	8.0	5.1	8.5	4.4
CaO	9.2	5.7	7.9	7.7	7.3
MgO	4.3	3.0	3.7	4.2	2.8
SrO	-	-	0.04	-	-
BaO	-	-	2.02	-	0.04
Na_2O	15.3	15.8	14.8	15.5	15.7
K_2O	0.8	0.8	0.9	1.1	1.4
Fe_2O_3	0.3	0.3	0.3	0.07	0.3
TiO_2	0.1	0.1	0.1	0.03	0.06
SO_3	0.1	0.1	0.7	0.07	0.21
F	-	-	-	0.7	-
k_{dis} (ng/cm^2/h)	2	600	150	300	100
Density (g/cm^3)	2.48	2.44	2.52	2.47	2.45

The other two fibers, MMVF 10 and MMVF 11, were obtained from the TIMA (Thermal Insulation Manufacturers Association) repository, and the compositions are those obtained from chemical analysis of these fibers as reported by Hesterberg et al. (1993). These fibers were tested recently in chronic inhalation studies at the Research and Consulting Company (RCC) in Geneva, Switzerland (Hesterberg et al., 1993).

Samples (70 mg) of each type of fiber were irradiated for 7.5 h in a total flux density of 1.5 x 10^{12} thermalized neutrons/cm^2/s in the University of London Imperial College nuclear reactor (Silwood Park, Ascot, Berkshire). This irradiation induces ^{24}Na in the ^{23}Na(n,gamma) -> ^{24}Na reaction. Sodlium-24 has a half-life of 15 h and emits 1.37- and 2.75-MeV gamma rays in 100% of disintegrations. Following irradiation, the activated fibers were suspended in a known volume of sterile physiological saline solution (0.9% weight/volume) to give stock suspensions of 6 mg/ml.

TABLE 2. Average and Standard Deviation of the Initial Fiber Length and Diameter Distributions (μm) and their Correlation Coefficient and Some of Their Properties

Statistic	X7779	X7753	X7484	MMVF 10	MMVF 11
Average diameter	1.81	1.99	2.31	1.11	1.42
Diameter SD	0.41	0.42	0.52	0.50	0.80
Average length	18.8	18.3	22.4	21.3	27.7

Length SD	10.6	12.4	11.5	18.9	23.8
Correlation coefficient	0.16	0.08	0.03	0.06	0.15
Number of fibers	806	398	446	487	599

Female Fischer 344 rats (Harlan Olac, Bicester, Oxfordshire), aged about 13 wk, were divided randomly into 5 groups of 25 animals. Rats from each group received 0.2 ml of stock fiber suspension, containing 1.2 mg of fibers, by intratracheal instillation under light halothane anesthesia. During the instillation procedure, after every sixth animal, a 0.2-ml sample of the fiber suspension was taken. This sample was passed through the same size of catheter as used for instillation into the rat trachea. These aliquots were subsequently diluted, and filter samples were prepared to enable fiber numbers and dimensions to be determined.

At 2 days after instillation, each rat was gamma counted with its thorax located centrally between coaxial NaI(TI) detectors to determine the retained ^{24}Na activity. Five rats from each group were then killed and the number of fibers remaining in their lungs determined following digestion with sodium hypochlorite solution (4% free chlorine) at 4°C for 4 h. The correlation between thoracic radioactivity and the number of fibers retained enabled estimates of the fiber numbers in the lungs to be made in all the other rats in that group. This procedure for estimating the number of glass fibers retained following their administration by intratracheal instillation has been described previously (Morgan et al., 1993).

Groups of typically 3 animals were killed at various times up to 1 yr after instillation by an injection of sodium pentobarbitone (Sagatal). After death, the lungs were excised and, after removal of the tracheal primary bifurcation, digested with hypochlorite solution at 4°C. Aliquots of the stock fiber suspensions taken at the time of instillation, and of each lung digest, were passed through 2.5-cm-diameter mixed cellulose ester filters (Millipore HA, pore size 0.45 μm), which were washed with 10 ml of deionized water and dried. In addition, a series of control filters was prepared by passing various Volumes of deionized water, and of the physiological saline used to prepare the fiber suspensions, through the same type of filter. These control filters were prepared and counted in the same manner as the lung digests, and were found not to have a significant number of fibers.

In order to determine whether the sodium hypochlorite digestion at 4°C affected the fibers, it was carried Out on fibers that had been partially dissolved in vitro. These tests showed no significant degradation of the fibers.

The filters were cleared using the acetic acid/dimethyl formamide technique (Le Guen & Galvin, 1981) for fiber counting and characterization. The cleared filters were examined by phase-contrast optical microscopy using a Nikon Microphot-FX Microscope fitted with a Nikon 4OX plan Ph3 objective and Bosch 2.5-cm Newvicon tube camera. Fibers in randomly selected fields were counted and measured using a Magiscan image analysis system and Genias 3.6 and Results 4.2 software (Applied Imaging, Gateshead). The length, area, and perimeter of each object were measured, and the diameter was calculated by dividing the object area by its length.

After measuring 30 randomly selected 170 x 170 μm fields on each filter, the number of fibers on the filter was estimated, assuming a sample area of 278 mm^2 on the cleared filter. The fiber number was then corrected for the volume of the aliquot filtered. The fraction of initially retained fibers remaining in the lung was computed by dividing this number by the estimated number of fibers retained at 2 days derived from the thoracic ^{24}Na activity of that animal.

Measurements of the dissolution of glass fibers in vitro have shown that the dissolution follows the rate law

$$dM/dt = -k_{dis} A \quad \textbf{(1)}$$

to a good approximation (Leineweber, 1984; Potter & Mattson, 1991; Scholze, 1988), where M is the mass of the fiber, A is the surface area in contact with the solution, t is the time, and k_{dis} is the dissolution rate constant. If the fibers are assumed to be cylinders with diameter D that decreases as dissolution proceeds, and the surface area of the ends is neglected, Eq. (1) has the solution

$$D(t) = D_0 - 2k_{dis} t/\rho \quad \textbf{(2)}$$

where ρ is the density of the fiber and D_0 is its initial diameter.

The diameter of each fiber in a batch of fibers with different diameters follows Eq. (2) until it decreases to zero. Therefore the peak diameter, or most probable diameter, obeys Eq. (2) also. Thus the peak diameter of a batch of fibers from a rat lung would be expected to decrease with time at a constant rate. However, it should be noted that the average diameter, whether arithmetic or geometric mean, or even the median, would not be expected to follow Eq. (2), since some fibers disappear. Thus the peak diameter is a good parameter with which to follow dissolution, whereas any sort of average diameter is not.

The number of fibers remaining in the lung was simulated by applying Eq. (2) to each fiber. The lengths and diameters for each fiber measured in the aliquots taken at the time of instillation were used as the initial fibers. Equation (2) was applied to each measured fiber to simulate the lengths and diameters of fibers present at subsequent times. Fibers that have dissolved completely are indicated by a zero or negative diameter in Eq. (2), and the number remaining can be compared with the relative number recovered from the lungs at that time.

RESULTS

The most direct evidence for dissolution of fibers in the lung is a reduction in their diameters. However, interpretation of changes in fiber diameter in vivo is complicated by the fact fibers may exist in different environments within the lung, each yielding a different dissolution rate. For example, most short fibers are likely to be contained within the cytoplasm of alveolar macrophages where the pH is lower than that of extracellular fluid (Morgan et al., 1982). Studies of the intraphagolysosomal pH of rat alveolar macrophages (Nyberg et al., 1989) give values ranging between 4 and 5. The dissolution of these types of glass fibers is much slower at this pH than in simulated lung fluid at pH 7.4 (Potter & Mattson, 1991). It is to be expected, therefore, that dissolution rates measured in vitro will approximate those observed for long fibers rather than short ones. When rounded up, the mean diameter of the rat alveolar macrophage is about 12 μm, but cells that contain fibers are generally larger than average and, in their normal configuration, can accommodate fibers up to about 20 μm in length (Morgan & Eastes, 1993, unpublished results).

In order to take account of differences in lung environments, the measured fibers were divided into 2 groups, those 20 μm or longer and those shorter than 20 μm. The diameters of the fibers in each of the 2 length groups were displayed in a histogram with 0.1-μm bin widths for each fiber type and time after instillation, including the instillation aliquot. The peak or most probable diameter was then taken from each histogram and plotted as a function of time after instillation in Figures 1-5 for the long and short fibers separately. In cases where two or more bins had the most observed values, or where the two highest differed by one, each of these diameter values was plotted. The dashed lines in Figures 1-5 represent the thinnest fiber that can be resolved reliably with the phase-contrast optical microscope system used. The solid lines in Figures 1-5 are the expected evolution of diameter from Eq. (2) using the value of k_{dis}, measured in vitro, given in Table 1.

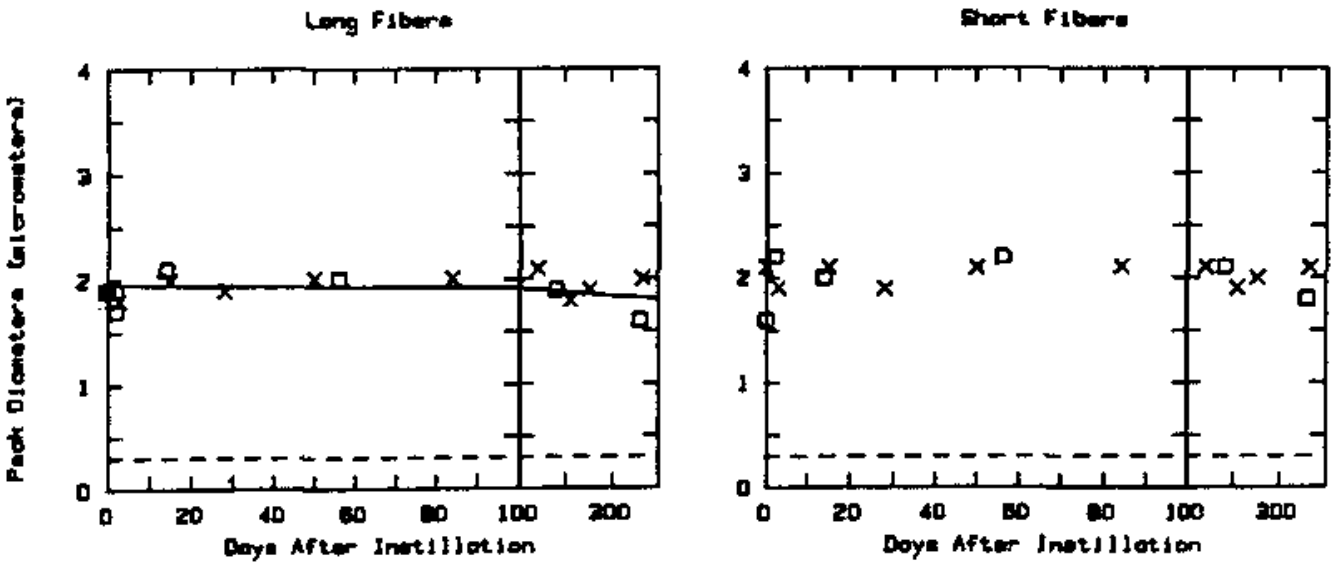

FIGURE 1. Peak or most probable diameter (x and squares from two independent Studies) of the X7779 fibers recovered from the animals' lungs at various times after instillation. The peak diameters of the set of long fibers, 20 jum or longer, and the short ones, less than 20 um, are plotted separately in the left and right panels, respectively, A compressed scale is used after 1 00 days.

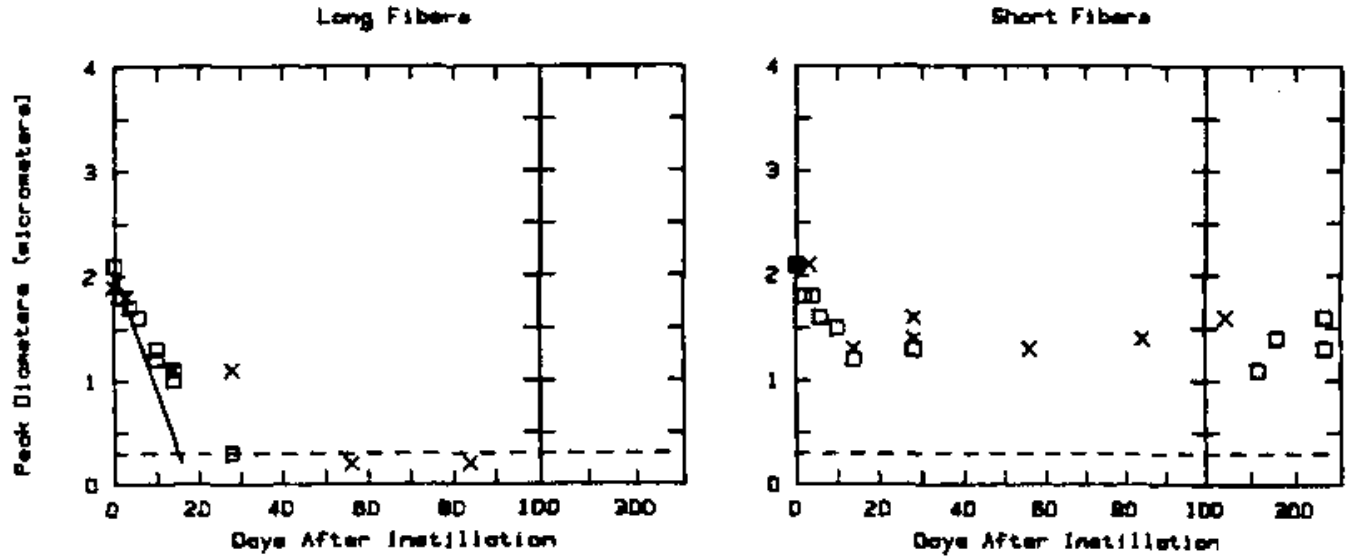

FIGURE 2. Peak diameter of X7753 glass fibers in vivo (x and squares from two independent studies). See Figure 1 for details.

The X7779 fibers shown in Figure 1 have such a low dissolution rate that no detectable change in fiber diameter would be expected over the duration of the study. The initial peak diameter of these fibers was about 2 μm, and it was almost unchanged after 1 yr in agreement with the solid line from Eq. (2). There was also no detectable change in the diameter of the short fibers (less than 20 μm long) as expected, since these would have dissolved even more slowly. Included in Figure 1 are the results of two separate experiments, performed over a year apart. The earlier study, shown by the symbols x, involved only the two fibers X7779 and X7753. The later study, denoted by the squares, used all five fiber types, and it agrees well with the earlier experiments.

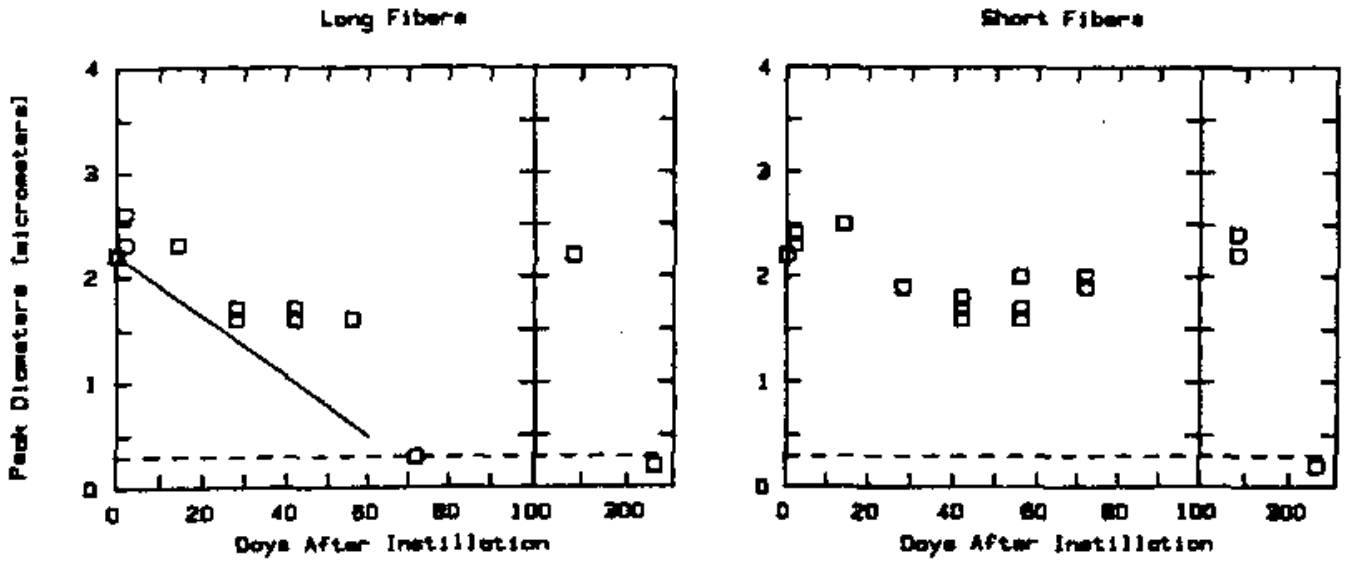

FIGURE 3. Peak diameter of X7484 glass fibers in vivo (squares). See Figure 1 for details.

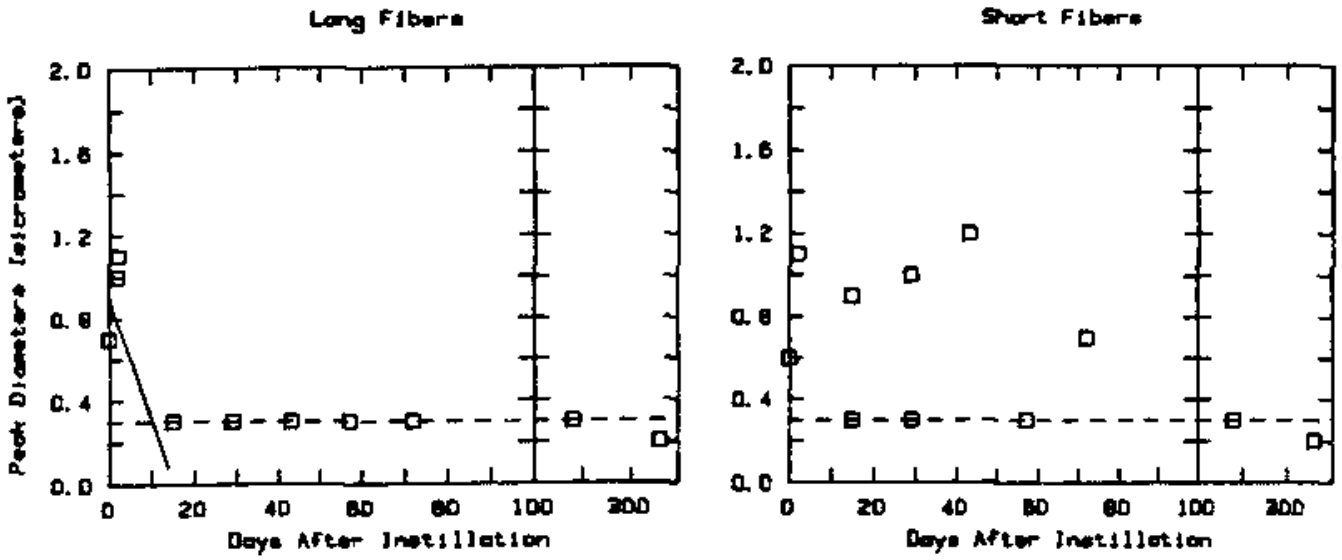

FIGURE 4. Peak diameter of the MMVF 10 glass wool fibers in vivo (squares). See Figure 1 for details.

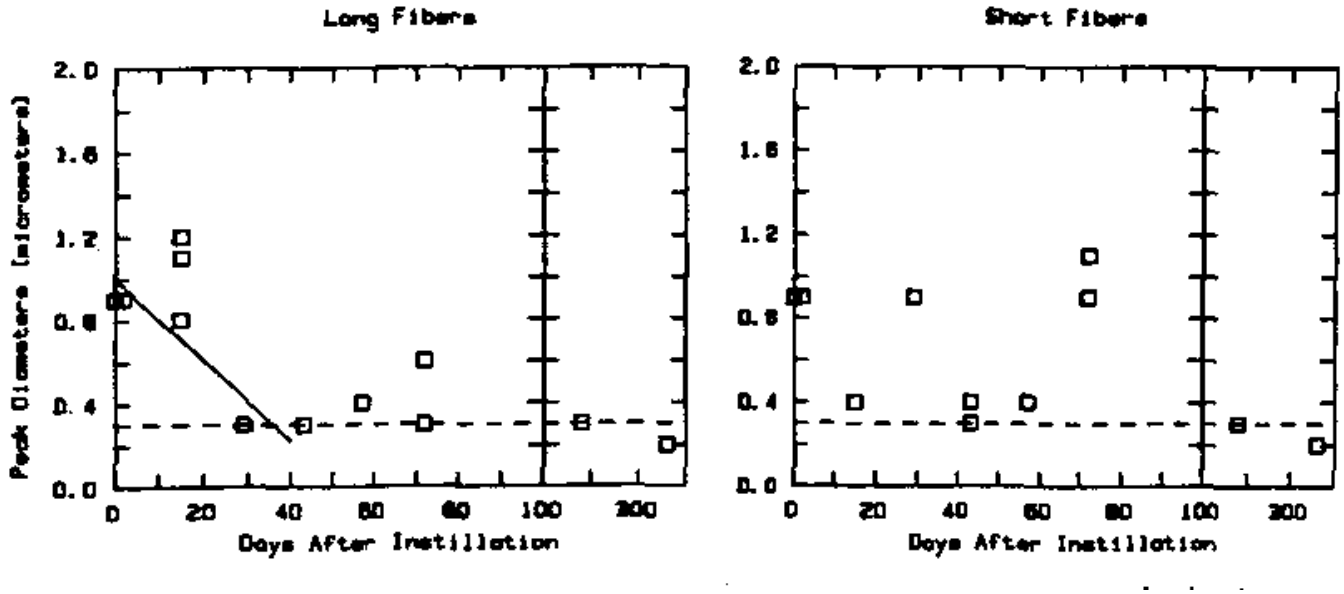

FIGURE 5. Peak diameter of the MMVF 11 glass wool fibers in vivo (squares). See Figure 1 for details.

The peak diameters for the rapidly dissolving X7753 fibers recovered from the lungs are shown in Figure 2. For the long fibers, over 20 μm long, the peak diameter decreased steadily until it reached the limit of resolution (about 0.3 μm), and remained there as long as there were enough long fibers to allow the location of a peak to be determined. Once again, the agreement between the in vivo data and the solid line calculated from the dissolution rate measured in vitro is excellent. Figure 2 also includes the results of two independent studies and, as in Figure 1 , these can be seen to be in good agreement. During the first 3 wk of the study, there was a decrease in the peak diameter of short X7753 fibers, which had the same slope as for the long fibers. However, after 3 wk, the peak diameter remained at about half the initial value for the remainder of the study.

The dissolution rate of fiber X7484 measured in vitro is intermediate between those of the X7779 and X7753 fibers. As shown in Figure 3, there is fairly good agreement between the peak diameters observed in vivo (square symbols) for the long fibers and the solid line predicted by Eq. (2), except for an anomalous point at 180 days. The peak diameters of the short fibers varied considerably, but there seems to be no definite trend with time.

The peak diameters of the MMVF 1 0 and MMVF 1 1 samples are shown in Figures 4 and 5, respectively. The average diameters of these samples are about half that of the other fibers and they are much less uniform in diameter. As a result, there is considerably more variation in the location of the peak diameter. Nevertheless there is good agreement between the peak diameters of the long fibers

measured in vivo (squares) and that expected from measurements in vitro (solid line). The slightly greater dissolution rate of MMVF 10 compared to MMVF 1 1 is clearly seen in vivo as well. There is no such trend for the short fibers.

The excellent agreement between the in vivo peak diameter of glass fibers with widely different dissolution rates and that derived from measurements in vitro, as shown in Figures 1-5, is evidence that a significant number of long fibers dissolve completely in animal lungs at the rate measured in vitro. It does not show, however, if dissolution is responsible for removing most of the long fibers or merely some of them. To answer that question, information is needed about the total number of fibers remaining in the lung after instillation.

The total number of fibers of all lengths remaining in the animals' lungs is shown in Figures 6 and 7 for fibers with the two extremes of dissolution rate, X7779 and X7753. The number of fibers is expressed as the percent of those present at the 2-day sacrifice. It is clear that the variation in these data is too great to allow the sort of quantitative comparison required to answer this question. In spite of the wide variation in fiber number, it is apparent in Figure 6 that there is no decrease in the number of slowly dissolving X7779 fibers as long as 1 yr after instillation. In contrast, there is a decrease in the total number of X7753 rapidly dissolving fibers (Figure 7), and it begins about 20 days after instillation, the time when the peak diameter for long fibers disappeared in Figure 2. It is also clear from Figure 6 that there is no detectable macrophage-mediated removal occurring via the mucociliary escalator, which would have been expected to clear at least some of the short fibers. Macrophage-mediated transport to the lymph nodes would not be detected in this study, as the lymphoid tissues were excised with the lungs.

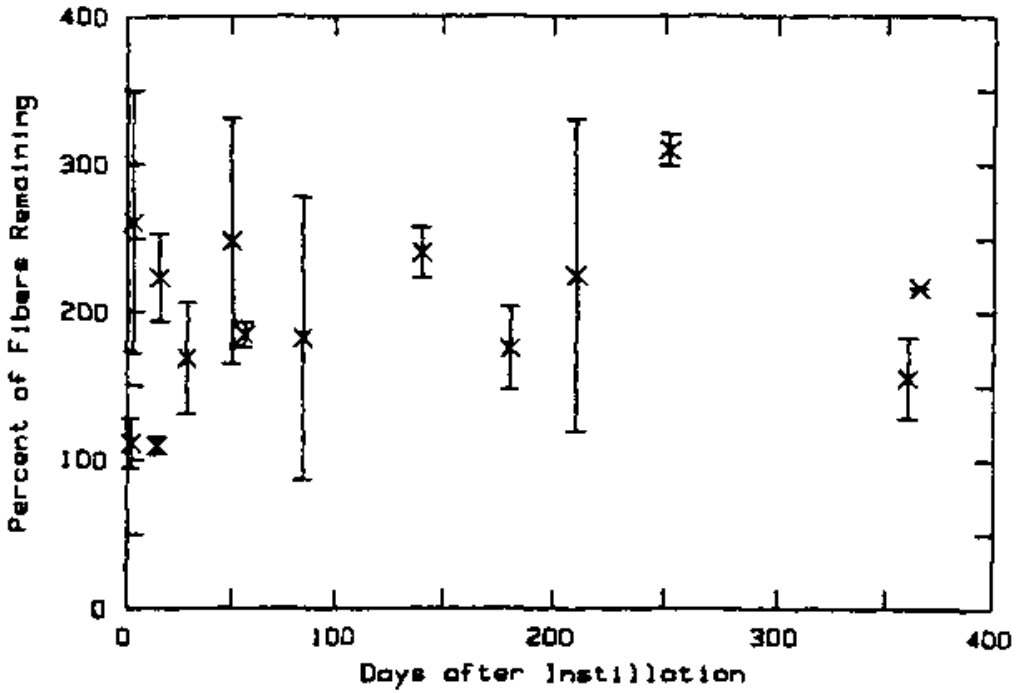

FIGURE 6. Total number of X7779 fibers remaining at Various times after instillation, expressed as the mean percent of those present at 2 days. The error bars are plus and minus one standard error of the mean.

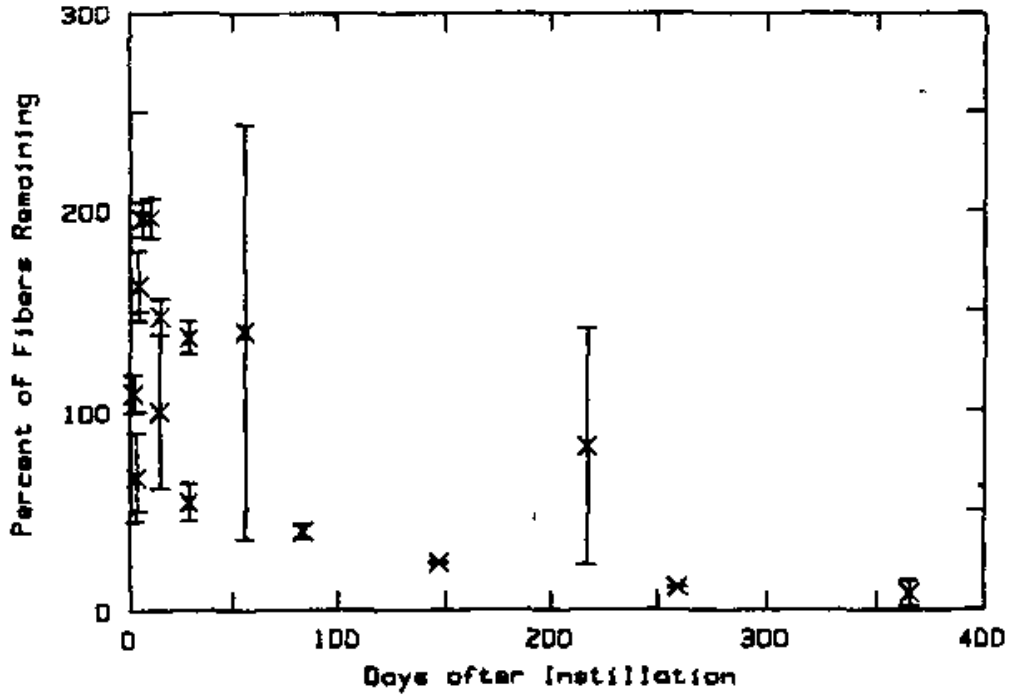

FIGURE 7. Total number of X7753 fibers remaining at various times after instillation, expressed as the mean percent of those present at 2 days. The error bars are plus and minus one standard error of the mean.

If it is assumed that no macrophage-mediated clearance of either short or long fibers happens after these intratracheal instillations, and the short fibers do not dissolve completely, as suggested by Figures 1-5, then the number of short fibers in a sample could be used as an indication of the total number of fibers originally present in the animals' lungs. Therefore the ratio of the number of long fibers (20 μm or longer) to short fibers (less than 20 μm) could be used as a guide to the number of fibers retained. Graphs of the ratio of long to short fiber number in the lung are shown in Figures 8-12 for each fiber type after instillation. The error bars are the standard error of the ratio, estimated from the number of long and short fibers counted. The solid line is calculated from the fiber length and diameter distribution of the initial aliquot, assuming that the long fibers dissolve by Eq. (2) and the short ones are unchanged.

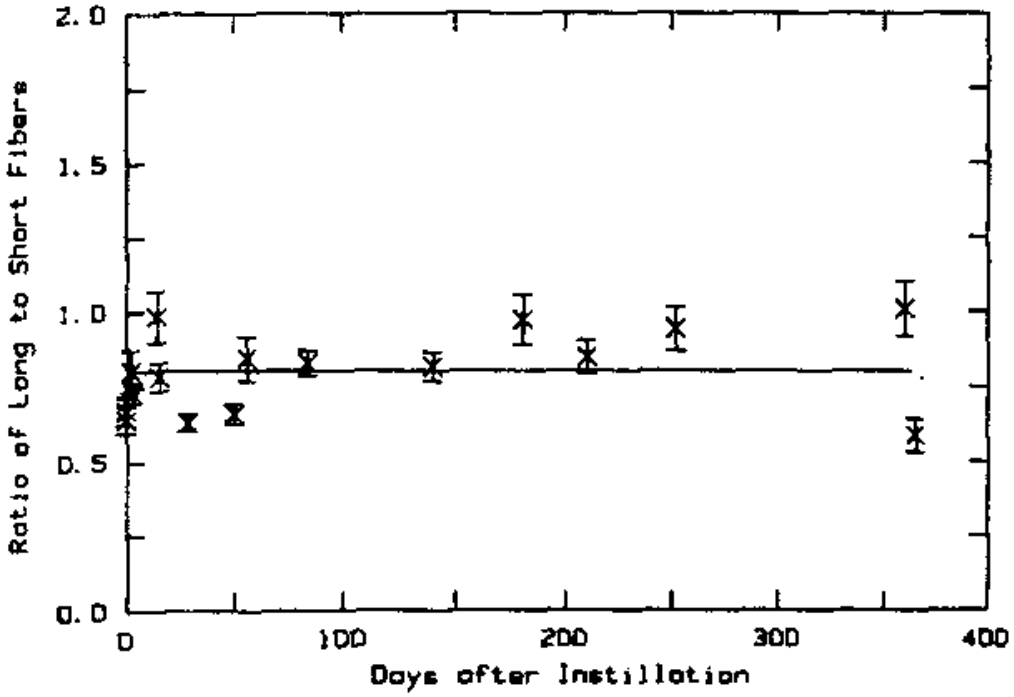

FIGURE 8. Ratio of the number of long fibers (20 jum or longer) to short fibers (less than 20 μm) for a sample of X7779 fibers removed from animals' lungs at various times after instillation (x symbols). The error bars are plus and minus one standard error, estimated from

the number of fibers in the numerator and denominator of the ratio. The solid line is the ratio calculated from the initial length and diameter distribution and a dissolution model.

The ratio of the number of long to short fibers for X7779, the slowly dis- solving fiber type, agrees well with the calculated line (Figure 8). Obviously, no mechanism operates to clear either the long or the short fibers. The scatter of the ratios would suggest that the error of the mean ratio plotted is at least twice as large as the error bars that are based only on the number of fibers counted.

Figure 9 shows the ratio of long to short fibers for the rapidly dissolving X7753 composition. There is good agreement between the values measured in vivo (x symbols) and that calculated by the dissolution model (line), within the variation observed in Figure 8.

Similar reasonable agreement between the in vivo ratio and the dissoluon model is observed for the X7484 glass fibers in Figure 10 and for the MMVF 10 and MMVF 11 glass wool fibers (Figures 1 1 and 12). In spite of the considerable scatter in the data, there is a definite tendency for the in vivo measured ratios to be somewhat larger than that predicted by the simple dissolution model. This discrepancy could be the result of some long fibers not dissolving quite as rapidly as the model predicts or from a removal of some short fibers by macrophage action, which is ignored in this model.

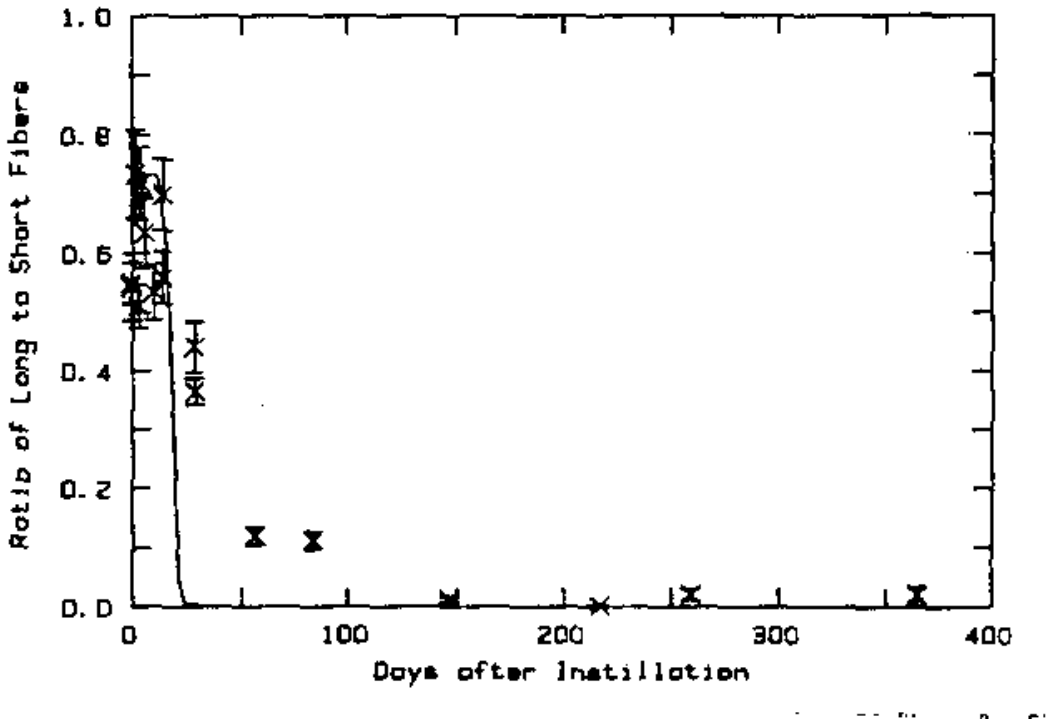

FIGURE 9. Ratio of the number of long fibers to short fibers for a sample of X7753 fibers. See Figure 8 for details.

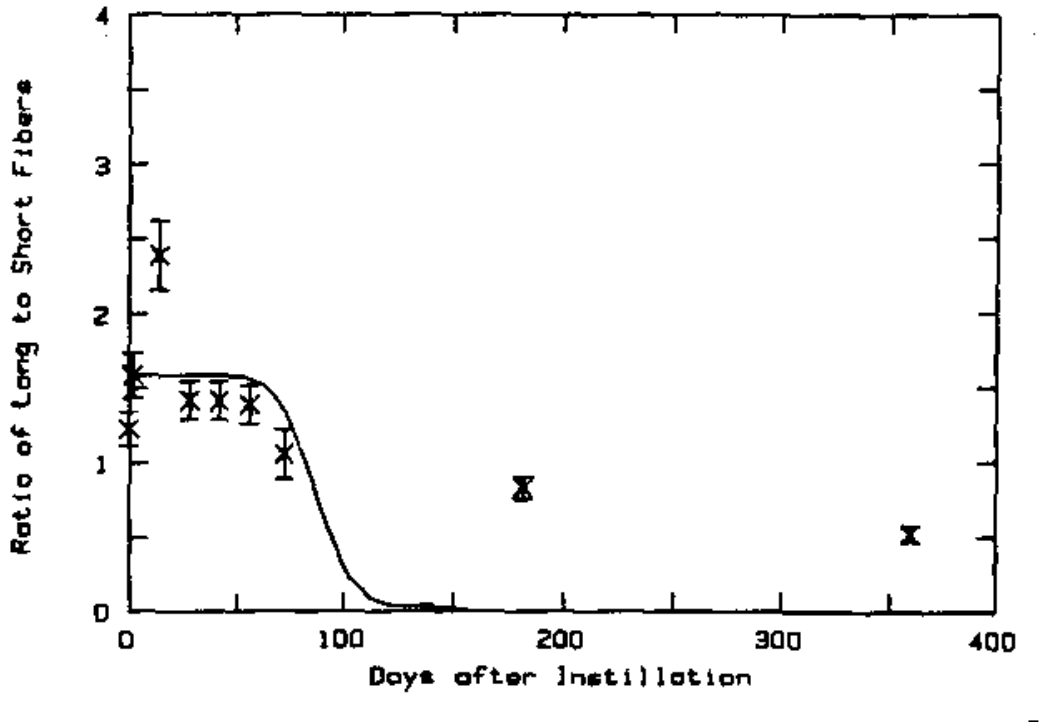

FIGURE 10. Ratio of the number of long fibers to short fibers for a sample of X7484 fibers. See Figure 8 for details.

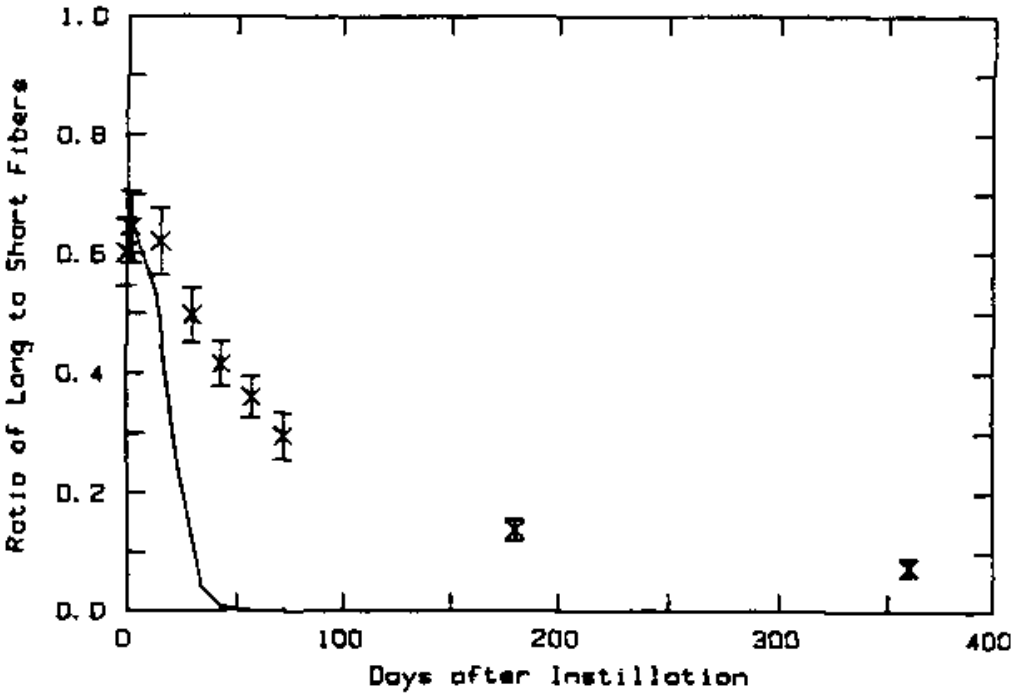

FIGURE 11. Ratio of the number of long fibers to short fibers for a sample of MMVF 10 glass wool fibers. See Figure 8 for details.

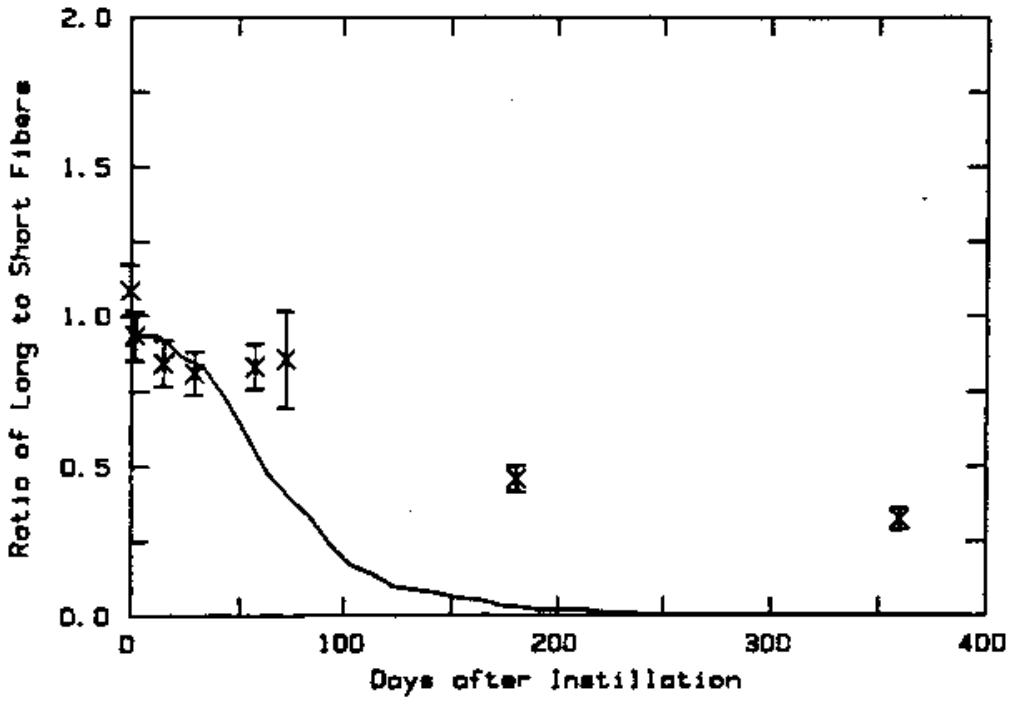

FIGURE 12. Ratio of the number of long fibers to short fibers for a sample of MMVF 11 glass wool fibers. See Figure 8 for details.

DISCUSSION

The evolution of the in vivo peak diameter of long fibers displayed in Figures 1-5 provides direct evidence that many fibers are dissolving in the rats' lungs. The excellent agreement between the in vivo peak diameter and that calculated from the dissolution rate measured in vitro shows that the long fiber dissolution is correctly simulated by the in vitro experimental arrangement. In addition, it is clear from Figures 1-5 that the dissolution of many long fibers proceeds by reducing the fiber diameter steadily until the fibers disappear. The effect is seen in glass fibers over a wide range of dissolution rates, from fibers that dissolve in 1 mo to those lasting several years.

Long fibers are defined here as those 20 μm or longer, merely for convenience. There is nothing in these data to suggest that some short fibers are not dissolving too, only that not enough of them are dissolving to dominate the fiber diameter distribution and to appear in the peak diameter.

Obviously, a significant number of long fibers dissolve completely in rats' lungs, but one would like to know whether most or all of the long fibers are cleared by dissolution. Estimates of the number of fibers remaining in the animals' lungs after intratracheal instillation were not precise enough to give this sort of detailed information (Figures 6 and 7). These estimates do suggest, however, that macrophage-mediated clearance via the conducting airways, which serves to clear at least the short inhaled fibers, did not operate after intratracheal instillation. In spite of the scatter of the fiber number data, it appears that the fastest dissolving-fiber was being removed from the lungs, whereas the slowest dissolving one was unaffected.

Macrophage-mediated removal of even the short fibers from the lungs was not observed in this study, although it has been seen following inhalation and in some previous intratracheal instillation experiments. On the basis of an analysis of particle clearance, Morrow has suggested that macrophagemediated clearance is impeded when the volume of deposited particles amounts to 25-90 μm^3 per available macrophage (Morrow, 1992). The 1.2 mg of glass fibers instilled in the present experiments is 19 μm^3 per macrophage, assuming an average number of 2.5 x 10^7 macrophages in the lungs of Fischer 344 rats (Morrow, 1992). Thus little impedance of clearance would be expected in the present study. Indeed, efficient macrophage-mediated clearance was observed following intratracheal instillation of 0.5 mg or 8 μm^3/macrophage of short glass fibers in a previous study (Morgan et al., 1982). On the other hand, there was no clearance of the same volume of 30-μm-long fibers in that same study, although there was a significant reduction in fiber diameter, indicating that dissolution had occurred (Morgan et al., 1982), in agreement with the present study. However, another intratracheal instillation study with as much as 20 mg or 320 μm^3/macrophage of short fibers did show clearance (Bernstein et al., 1980). One possible reconciliation of these conflicting results may lie in the fact that the present study used a mixture of both long and short fibers. The long fibers may have occupied the available macrophages, limiting their ability to remove both the long fibers as well as the short fibers. Another possible explanation for these discrepancies Could be that the present intratracheal instillation procedure deposited the fibers in a relatively small region of the lung, yielding a locally greatly increased volume of fibers per available macrophage. The local fiber burden may have been enough to overload the lung in the region where the fibers were deposited.

Better evidence for the number of long fibers cleared by dissolution comes from the ratio of the number of long fibers to the number of short fibers remaining in samples removed from the rats' lungs at various times after instillation, shown in Figures 8-12. The ratio of long to short X7779 slowly dissolving fibers remained constant for 1 yr in the presence of considerable scatter, whereas the long X7753 rapidly dissolving fibers disappeared rapidly. There was reasonable agreement between the in vivo measured ratios of long to short fibers (x symbols) and that calculated from the fiber length and diameter distributions measured in the initial aliquot for a simple dissolution model (solid line). This model assumed that each fiber 20 um or longer dissolved according to Eq. (2) and disappeared when the calculated diameter reached zero, and that all shorter fibers were Unaffected. This model is clearly naive, since one does not expect a sharp cutoff at 20 μm or at any other length in the fibers that can be enveloped by macrophages. If some long fibers were at least partially involved in macrophages, these would not dissolve as rapidly as the model would predict. Likewise, if macrophage-mediated clearance were to Occur for some short fibers, it Would increase the ratio of long to short fibers above that predicted by this model. One or both of these possibilities may be occurring in the fibers studied here, especially in MMVF 10 (Figure 11) and to some extent in the other fibers. Although the in vivo measured points (x symbols) do not often deviate from the calculated solid line by much more than the scatter exhibited for X7779 in Figure 8, there is a definite trend toward higher ratios in vivo than calculated. The trend is more pronounced when the most fibers have been removed. Thus it is possible that some macrophage-mediated clearance occurs, as the effect of overload is reduced 3 mo or more after intratracheal instillation of the faster dissolving fibers. It would serve to increase the ratio of long to short fibers above what the model would predict, as observed in Figures 9-12.

It is interesting that fiber breakage cannot explain the results of this study. Even though the number of fibers appears to increase by more than a factor of two in the weeks following instillation, it is not accompanied by a corresponding decrease in average length or diameter as it would be if fiber breakage or fragmentation were the cause (Figures 6 and 7). The average length and diameter of the fibers do not change during the time that the number of fibers has apparently doubled. The erratic behavior of the measured fiber numbers and the wide error bars suggest that these excursions are the result of random variations in the small number of rats, typically three, in each group.

Fiber breakage also cannot explain the deviations from the calculated curves (solid lines) in Figures 9-12. Fiber breakage would decrease the number of long fibers and Simultaneously increase the short fiber count, thus decreasing the ratio of long to short fibers below what dissolution alone would predict. If anything, the observed ratios are larger than the dissolution calculation predicts, not smaller. Therefore it is clear that fiber dissolution, not breakage, is the main contribution to the elimination of the long fibers.

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