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Influence of Well-Defined Mineral Fibers on Proliferating Cells

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The effects of well-defined asbestos and man-made mineral fibers, as well as glass and synthetic fluoroamphibole, on phagocytizing permanent rat tumor cells were tested. The following parameters were compared: cell proliferation as determined by cell count and ^{3}H-thymidine incorporation, RNA synthesis by ^{3}H-uridine uptake, protein synthesis by incorporation of ^{3}H-labeled amino acids, protein content and plasma membrane permeability by release of lactic dehydrogenase.

The dosage of most of the dusts was estimated gravimetrically, but for some dusts also numerically. Because of the wide range of different fibers lengths, diameters and specific weights, it was sometimes difficult to compare chemically and physically differing fiber fractions with the same fiber counts. In some cases, resulting weights are so different that a direct comparison of the conclusions is impossible. The results with fibers of diverse sources showed the same trends: the toxicity of fibers increases with increasing length and dose. In this test system we found an inhibition of DNA and RNA synthesis. Protein synthesis as measured by amino acid uptake per total cell culture decreased, but the protein content of the single cell increased as determined by the Lowry method.

The increase of plasma membrane permeability as determined by lactic dehydrogenase was also dependent on fiber length and concentration. Generally the thinner the fiber, the greater the toxicity when gravimetrical dosage and the same length distributions are employed.

Beyond that we can state that the toxicity of fibers from different sources with similar fiber dimensions is similar. One of the glass fiber fractions has a comparable geometry (length, diameter) to the UICC fraction of chrysotile and exhibits the same high toxicity.

Introduction

The geometry of inhalable mineral fibers influences their biological effects (*1-6*) and is probably one of the most important factors for the pathogenicity of fibrogenicity and carcinogenicity. Additional factors in influencing the biological effects of different fibers are elasticity, surface loading, chemical composition, adsorption capacity for inorganic and organic molecules and the solubility in the organism. The exact dimensions of the fibers of interest are still unclear. Therefore it seemed essential to investigate the length-dependent toxicity of asbestos fibers and man-made mineral fibers in comparison.

Cell tests with asbestos and man-made mineral fibers of three different lengths (*7*) were done earlier (*8, 9*). The results show a dose and fiber length-dependent toxicity. These fiber fractions were not or only partially comparable regarding diameter. Further investigations were carried out by Brown and Davies et al. (*10-12*).

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However, for direct comparison of different fiber types, it is necessary to have a number of fractions, well characterized by length, diameter, chemical composition, and number of fibers per weight.

Material and Methods

Dusts

Table 1 gives the exact distributions of length and diameter of 15 different fiber fractions prepared and characterized by the Johns Manville Corp. Six of these are glass fibers, two synthetic are fluoroamphiboles, four are chrysotile, and three are crocidolite. The fiber fractions have been separated by length and diameter. The fiber fractions were characterized by an approximate description: long and short, thick, thin and very thin. (Table 1).

After weighing and after sterilization by dry heat, the fibers were suspended in MEM by ultrasound.

Cell Cultures

Phagocytic epitheloid acites tumor cells induced in Wistar rats 8 months after intraperitoneal admin-

Table 1. Length and diameter distributions of fibers.

Dust no.	Fiber type	Length distribution: Length, μm	Length distribution: % of fibers	Diameter distribution: Diameter, μm	Diameter distribution: % of fibers
1	Short, very thin glass fibers (4106-23-7)	<1	83.8	<0.1	52.0
		1.0-1.9	11.5	0.1-0.14	35.4
		>1.9	4.8	0.15-0.19	5.7
				0.2-0.29	6.9
2	Long, very thin glass fibers (4106-24-2)	1.0-2.9	9.2	<0.1	63.7
		3.0-4.9	15.8	0.1-0.14	25.6
		5.0-9.9	25.0	0.15-0.19	8.8
		10-19	30.0	0.2-0.29	1.6
		20-29	9.2	0.3-0.59	0.3
		30-59	10.8	(0.41)	
3	Short, thin glass fibers (4106-4-3)	1.0-1.9	14.1	0.15	5.9
		2.0-2.9	35.2	0.15-0.29	16.4
		3.0-4.9	35.2	0.3-0.59	35.4
		5.0-9.9	8.5	0.6-0.99	26.2
		10-22	7.0	1.0-1.99	16.1
4	Long, thin glass fibers (4106-19-2)	<5	0.7	<0.15	51.4
		5.9-9	7.0	0.15-0.19	19.6
		10-19	37.5	0.20-0.29	11.9
		20-39	36.0	0.30-0.39	6.1
		40-59	14.8	0.40-0.59	6.2
		60-99	4.0	0.60-1.49	4.8
5	Short, thick glass fibers (4106-6-1)	1.0-2.9	22.4	<0.4-0.59	3.4
		3.0-4.9	33.5	0.6-0.99	5.8
		5.0-9.9	31.5	1.00-1.49	15.2
		10-19	10.5	1.50-1.99	28.6
		20-29	2.1	3.00-3.95	17.6
6	Long, thick glass fibers (4106-19-1a)	3.0-4.9	9.9	<0.2-0.59	4.1
		5.0-9.9	14.6	0.6-0.99	18.0
		10-19	28.9	1.0-1.49	35.2
		20-29	20.1	1.5-1.99	22.7
		30-59	22.1	2.0-2.95	14.8
		60-99	4.4	3.0-5.95	5.2
7	Jeffry fibrils (4173-46-1)	<1.0	29.5	<0.03-0.05	99.9
		1.0-1.9	34.7		
		2.0-4.9	27.6		
		5.0-9.9	6.6		
		10-19	1.6		
8	Jeffry fibrils (4173-46-2)	0.10-0.99	11.8	0.10	99.9
		1.0-1.9	20.0		
		2.0-4.9	24.1		
		5.0-9.9	20.5		
		10-19	17.7		
		20-39	5.9		
9	Short Munroe fibrils (4173-60-1)	0.40-9.99	22.5	0.03-0.05	99.9
		1.0-1.49	29.7		
		1.5-1.99	21.0		
		2.0-2.99	15.3		
		3.0-4.99	10.0		
		5.0-9.99	1.7		

Table 1. Length and diameter distributions of fibers.

Dust no.	Fiber type	Length distribution: Length, μm	Length distribution: % of fibers	Diameter distribution: Diameter, μm	Diameter distribution: % of fibers
10	Long Munroe chrysotile (4173-60-2)	3.0	0.0	<0.1	40.2
		3.0-4.9	0.7	0.1-0.29	20.2
		5.0-9.9	4.3	0.3-0.59	8.8
		10-19	26.4	0.6-0.99	13.6
		20-60	65.7	0.1-1.99	4.2
		60-80	2.9	2.0-2.5	13.3
11	Short crocidolite (S. African)	0.40-0.99	17.6	0.03-0.04	1.2
		1.0-1.9	44.4	0.05-0.09	7.5
		2.0-4.9	19.8	0.10-0.19	43.3
		5.0-9.9	6.2	0.20-0.39	32.4
		10-19	1.6	0.40-0.59	4.4
		20-29	0.4	0.60-0.99	1.2
12	Short crocidolite (4106-23-9)	0.1-0.69	47.8	<0.05-0.09	25.3
		0.7-0.99	22.3	0.10-0.14	40.0
		1.0-2.9	24.6	0.15-0.19	19.4
		3.0-4.9	3.5	0.20-0.29	13.3
		5.0-9.9	1.5	0.30-0.39	2.0
		10-19	0.3		
13	Long crocidolite (4106-22-4)	3.0-4.9	4.2	0.10-0.14	27.8
		5.0-9.9	13.6	0.15-0.19	15.0
		10-19	28.3	0.20-0.29	30.9
		20-29	23.0	0.30-0.39	13.1
		30-59	24.1	0.4-1.49	7.9
		60-129	6.8		
14	Short fluoroamphile (4106-31-3)	<1	89.9	0.03-0.09	46.0
		1.0-1.9	5.8	0.10-0.19	40.9
		2.0-4.9	3.6	0.20-0.39	9.6
		5.0-19.0	0.75	0.40-0.99	2.9
				1.0-2.95	0.6
15	Respirable fluoroamphibole (4106-30-1)	0.5-1.49	8.2	0.03-0.04	44.7
		1.5-2.9	21.5	0.05-0.14	25.3
		3.0-4.9	27.0	0.15-0.39	20.9
		5.0-9.9	27.5	0.40-0.99	5.6
		10-19	12.5	1.0-2.0	3.5
		20-39	3.5		

istration of nemalite were employed as a test system.

The cells were incubated with the fibers in suspension. For determination of cell counts and lactic dehydrogenase (LDH), release, tests were done in 25 cm^2 Falcon flasks. At different times the supernatants were photometrically examined for LDH. Protein content was measured by the Lowry method after cell lysis, and cell numbers were determined directly in the culture flask by counting five defined areas and calculating with a conversion factor.

For the investigation of DNA, RNA and protein synthesis by incorporation of radionucleides, the cells were incubated in microtiter plates with flat bottoms (Falcon Plastics) in the same concentrations as in the culture flasks. Two hours before the end of the incubation period, 20 μL of ^{3}H-labeled thymidine, uridine or an amino acid mixture was added (0.5 μCi).

For termination of marker incorporation and cell harvesting, 100 μL of mixture of neutral protease (Dispase, Boehringer, Mannheim, FRG) and a 1000-fold dose of cold thymidine, uridine or 3×10^{-3} NaN_3 in the case of protein synthesis were applied to each cavity. After half an hour at 37°C, the cells were collected on glass fiber filters with a Skatron cell harvester.

Incorporation was determined at 48, 72 and 96 hr by scintillation counting and expressed in counts per minute (cpm)

Results and Discussion

Figure 1 shows the cell counts as percent of con-

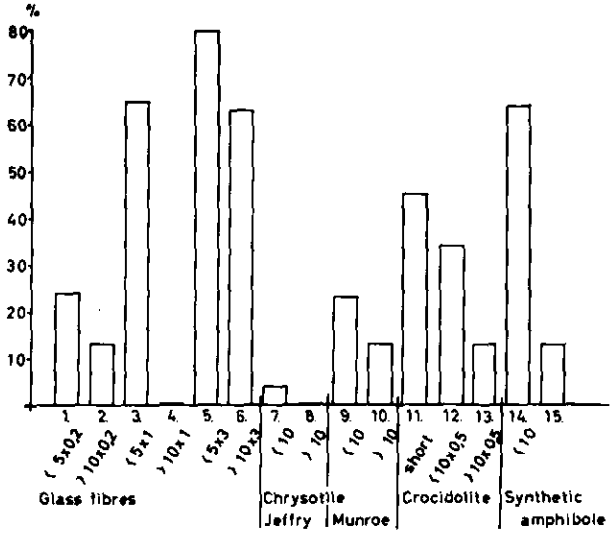

FIGURE 1. Number of cells (as % of control) 96 hr after incubation of proliferating cells with 30 μg dust/mL.

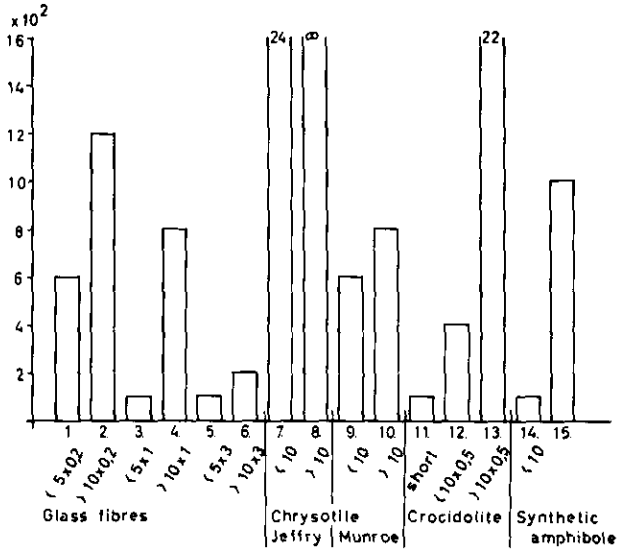

FIGURE 2. LDH release (as % of control) 96 hr after incubation of proliferating cells with 30 μg dust/mL (control = 100%).

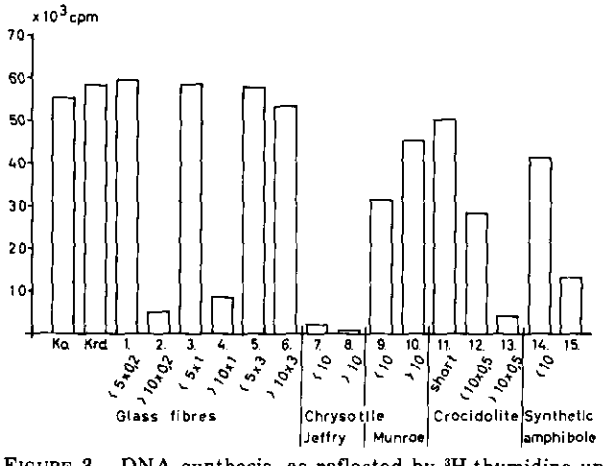

FIGURE 3. DNA synthesis, as reflected by ^{3}H-thymidine uptake by phagocytic tumor cells incubated with 15 different fiber dusts in a concentration of 15 μg/mL. Graph shows total counts for 48-96 hr. Cell concentration at the beginning of the test = 0.15 × 10⁶/mL.

trol 96 hr after the beginning of incubation. In all fiber types a length-dependent toxicity can be observed. Futhermore, one can see that the diameter in the glass fiber fractions also influences the cell toxicity.

Moreover, Jeffry chrysotile fractions and the long and thin glass fibers were found to be the most toxic samples, while the thick glass fibers as well as the short synthetic amphiboles proved only slightly cytotoxic.

Similar results were obtained with LDH release. As in the cell determinations, Jeffry chrysotile showed also the highest toxicity in terms of enzyme liberation. Both parameters demonstrated a significant toxic effect when cells were exposed to short and very thin glass fibers. In the quantification of DNA, RNA and protein synthesis, smaller effects could be detected; these cellular functions are inhibited only to a minor degree. Microscopic examination suports this finding with the observation of two to five nucleated cells. This could also be observed with the thick Munroe chrysotile fibers. The inverse results are observed with regard to DNA and RNA synthesis: the long synthetic amphibole fibers, have no or little influence on RNA synthesis and produced a significant decrease of DNA synthesis, in accord with the depression of cell number and LDH release.

Interesting also are the results with Munroe chrysotile: there is little influence on DNA and RNA synthesis, but high toxicity as measured by protein synthesis and cell counts at the end of the culture period. This finding can be understood by morphological examination: with dust 10 and partly with dust 12 we find a high proportion of cells with two or more nuclei. At the same time, protein content per cell increases, which agrees with the microscopic picture.

In contrast to our earlier experiment with the fibers fractions prepared by Dr. Spurny, with thicker fibers and also in the short fiber fractions a high toxicity is observed. In the earlier experiment, short fractions of glass fibers, chrysotile, crocidolite and amosite showed little or no cell toxicity.

In conclusion, in general the longer the fiber, the greater the toxicity. When using a comparable fiber length distribution one can furthermore say that the thinner the fiber, the greater the toxicity.

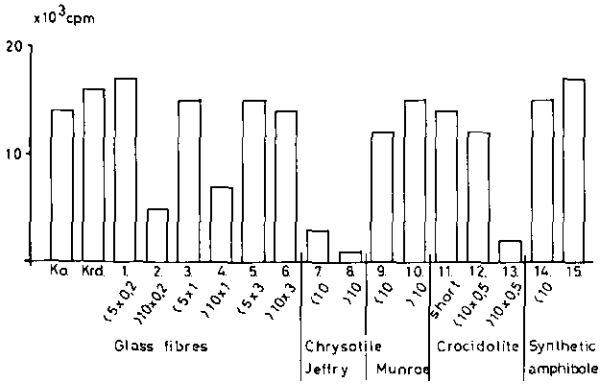

FIGURE 4. RNA synthesis, as reflected by ^{3}H-uridine uptake, by phagocytic tumor cells incubated with 15 different fiber dusts in a concentration of 15 μg/mL. Graph shows total counts for 48-96 hr. Cell concentration at the beginning of the test = 0.15 × 10^6/mL.

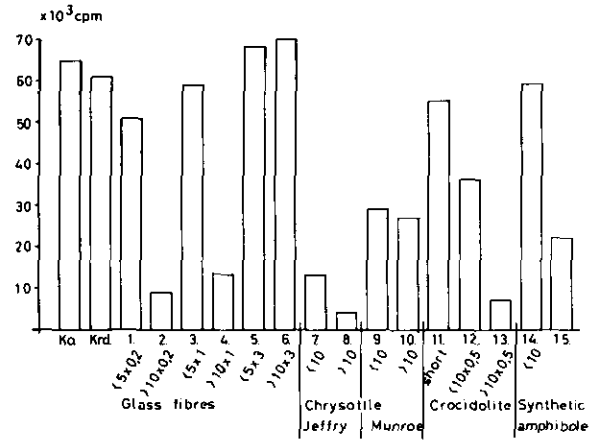

FIGURE 5. Protein synthesis, as reflected by uptake of ^{3}H-labeled amino acids by phagocytic tumor cells incubated with 15 different fiber dusts in a concentration of 15 μg/mL. Graph shows total counts for 48-96 hr. Call concentration at the beginning of the test = 0.15 × 10^6/mL.

Fractions of long and short fibers with a diameter of about 3.0 μm or with only a small proportion of fibers under 1.0 μm are nontoxic in this test system. The measurement of DNA, RNA and protein synthesis in combination of cell counts seemed to show a different influence of all mitosis by different fibers.

A problem in the evaluation of the fiber fractions is the dose or the type of dose: gravimetrical or numerical? The thinner the fiber, the more fibers are contained per weight. In different fiber fractions with a wide range of diameters it is virtually impossible to compare results according to the numerical dose because the difference in the dust loading of the single cell is very great. For direct comparison, one can only consider fibers with a similar diameter distribution.

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