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 ³H-thymidine incorporation, RNA synthesis by ³H-uridine uptake, protein synthesis by incorporation of ³H-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 fiber 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 gravimetric 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.

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-

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| Dust no. | Fiber type                | Length distribution | Diameter distribution |
|---------|---------------------------|---------------------|----------------------|
|         |                           | % of fibers         | % of fibers          |
|         |                           | Length, μm          | Diameter, μm         |
| 1       | Short, very thin glass fibers | 83.8                | 52.0                 |
| (4106-23-7) | 1.0-1.9                   | 11.5                | 35.4                 |
|         |                           | 4.8                  | 5.7                  |
|         |                           | >1.9                 | 2.0-0.29             |
| 2       | Long, very thin glass fibers | 9.2                  | 63.7                 |
| (4106-24-2) | 1.0-2.9                   | 15.8                | 25.6                 |
|         |                           | 4.8                  | 8.8                  |
|         |                           | 1.0-1.9              | 0.2-0.29             |
|         |                           | 3.0-4.9              | 0.3-0.59             |
|         |                           | 5.0-9.9              | (0.41)               |
| 3       | Short, thin glass fibers  | 14.1                | 5.9                  |
| (4106-4-3) | 1.0-1.9                   | 35.2                | 16.4                 |
|         |                           | 35.2                | 35.4                 |
|         |                           | 5.5                  | 26.2                 |
|         |                           | 7.0                  | 16.1                 |
| 4       | Long, thin glass fibers   | 0.7                  | 51.4                 |
| (4106-19-2) | <5                       | 7.0                 | 19.6                 |
|         |                           | 37.5                | 11.9                 |
|         |                           | 36.0                | 6.1                  |
|         |                           | 14.8                | 6.2                  |
|         |                           | 4.0                  | 4.8                  |
| 5       | Short, thick glass fibers | 22.4                | 3.4                  |
| (4106-6-1) | 1.0-2.9                   | 35.5                | 5.8                  |
|         |                           | 31.5                | 15.2                 |
|         |                           | 10.5                | 28.6                 |
|         |                           | 2.1                  | 17.6                 |
| 6       | Long, thick glass fibers  | 9.9                  | 4.1                  |
| (4106-19-1a) | 3.0-4.9                   | 14.6                | 18.0                 |
|         |                           | 28.9                | 35.2                 |
|         |                           | 20.1                | 22.7                 |
|         |                           | 22.1                | 14.8                 |
|         |                           | 4.4                 | 5.2                  |
| 7       | Jeffry fibrils            | 29.5                | 99.9                 |
| (4173-46-1) | <1.0                      | 34.7                | 99.9                 |
|         |                           | 27.6                | 99.9                 |
|         |                           | 6.6                 | 99.9                 |
|         |                           | 1.6                 | 99.9                 |
| 8       | Jeffry fibrils            | 11.8                | 0.10                 |
| (4173-46-2) | 0.10-0.99                 | 11.8                | 99.9                 |
|         |                           | 20.0                | 99.9                 |
|         |                           | 24.1                | 99.9                 |
|         |                           | 20.5                | 99.9                 |
|         |                           | 17.7                | 99.9                 |
|         |                           | 5.9                 | 99.9                 |
| 9       | Short Munroe fibrils      | 22.5                | 0.03-0.05            |
| (4173-60-1) | 0.40-0.99                 | 22.5                | 99.9                 |
|         |                           | 29.7                | 99.9                 |
|         |                           | 21.0                | 99.9                 |
|         |                           | 15.3                | 99.9                 |
|         |                           | 10.0                | 99.9                 |
|         |                           | 1.7                 | 99.9                 |
CYTOTOXICITY OF FIBROUS MINERAL DUSTS

Table 1. Length and diameter distributions of fibers.

| Dust no. | Fiber type          | Length distribution                      | Diameter distribution          |
|----------|---------------------|------------------------------------------|---------------------------------|
|          |                     | Length, μm          | % of fibers       | Diameter, μm          | % 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               | 28.4             | 0.6-0.99            | 13.8              |
|          |                     | 20-60               | 65.7             | 0.1-1.99            | 4.2               |
|          |                     | 60-80               | 2.9              | 2.0-2.5             | 19.3              |
| 11       | Short crocidolite (S. African) (4106-23-9) | 0.4-0.99            | 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 fluoroamphile (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           | 5.5               |
|          |                     | 10-19               | 12.5             | 1.0-2.0             | 3.5               |
|          |                     | 20-39               | 3.5              |                     |                   |

Registration 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 cm² 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 radionuclides, 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 ³H-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 × 10⁻⁸ NaNO₃ 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-
control 96 hr after the beginning of incubation. In all fiber types a length-dependent toxicity can be observed. Furthermore, 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 supports 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.
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: gravimetric 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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