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Tumorigenic Effect of Fibrous Dusts in Experimental Animals

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Fibrous dusts (chrysotile, glass fibers, nemalite, palygorscite, and gypsum) and granular dusts (actinolite, biotite, hematite, pectolite, sanidine, and talcum) were injected intraperitoneally into rats. The fibrous dusts (other than gypsum) resulted in a high incidence of mesothelioma (30 – 67%). Gypsum produced only 5% and granular dusts none at all. It is suggested that the fibrous shape leads to a high multiplication rate of cells and predisposes to tumor formation. Fibrosis, in the other hand, does not so predispose. Milled chrysotile with 99.8% fibers than 5 μm in length are carcinogenic in our experience. The carcinogenicity of glass fibers in our experiments may have significance for occupational situations.

The starting point of our investigations was the question whether the tumorigenic effect of asbestos fibers depends on physicochemical properties of the fiber or the shape of the fibers which are characteristic for all kinds of asbestos. For this purpose chemically different fibrous forms were compared to chemically similar dusts having different forms.

Dusts Tested

Tables 1 and 2 list the dusts tested in the animal experiments with respect to their chemical composition, particle shape, fiber length, and particle size. The fiber length and particle size were estimated by evaluation of electron micrographs.

Experimental Methods

The dusts were injected intraperitoneally in Wistar rats. We could not see any difference between the reaction of peritoneum and pleura. In addition, the injection did not essentially disturb the general status of the animals.

Table 1. Estimation of fiber length of fibrous dust by Electron microscopy.

Dust	Chemical composition	Fiber length	
		<2μm,%	<5μm,%
Chrysotile A	Mg silicate	78.7	93.9
Chrysotile (milled)	Mg silicate	97.4	99.8
Glass	Na, Ca borosilicates	49.9	72.6
Gypsum	Ca sulfate	65.0	75.0
Nemalite	Mg hydroxide	91.5	96.4
Palygorscite	Mg, Al silicates	37.5	70.0

Table 2. Estimation of particle size of grain dusts, by electron microscopy.

Dust	Chemical composition	Particle size	
		<2 μm, %	<5 μm, %
Actinolite	Ca, Mg, Fe silicates	a	a
Biotite	K, Fe, Mg, Al silicates	86.5	96.3
Hematite	Fe oxide (precipitated)	a	a
Hematite	Fe oxide (mineral)	a	a
Pectolite	Ca, Na silicates	86.2	98.4
Sanidine	K, Al silicates	85.6	97.2
Talc	Mg silicate	a	a

[a] No particle size analysis possible.

The best experimental method would be the inhalation of the dusts, but this method leads to

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enormous technical difficulties. The time required to produce tumours following injection of dusts is already long. This time would be prolonged tremendously by an inhalation method, since it takes months to get an effective dose at the vulnerable cell structures. The necessary concentration time of the dusts and the time to produce the tumors may even exceed normal life span.

The test dusts were suspended in saline solution at concentrations up to 25 mg/2 ml. For higher dosages, the injections were repeated once a week. The different test groups consisted 40 rats. Pure saline was injected in 80 control animals. The rats were observed until spontaneous death or sacrifice. All tumors were studied histologically.

Results

The UICC standard asbestos as well as palygorscite, nemalite, and glass fibers induced tumors in the abdominal cavity of 30–67% of the rats. Several dusts chemically closely related to chrysotile (like actinolite, biotite, pectolite, talcum), but of granular or platy shape with only a few fibers did not lead to the development of tumors except for a few cases. Calcium sulfate (gypsum) did show a fibrous shape but dissolved in the animal tissue and induced tumors only in 5% of the animals. Table 3 shows the tumor rate within the different experimental groups. Histologically nearly all the tumors were sarcomatous mesotheliomata.

In current experiments the lowest effective dosage of glass fibers was 2 mg/rat. The glass fibers were uncoated. The average diameter of the glass fibers was about 0.5 μm. With this dosage the first mesotheliomata occurred at 17 months. It seems of importance that 2 mg of glass fibers induced only slight adhesions around the liver lobes; 10 mg led to slight or medium adhesions and fibrous alterations, especially around the liver and the stomach; 50 mg of fiber dust induced tremendous adhesions on all abdominal organs with strong fibrosis. The fibrotic alterations generally could be differentiated from the numerous tumors. With 100 mg glass fiber only 6.5 months were necessary for tumor induction in the first rat.

Activity of Fibers within the Tissues

We are confronted with the question: Why do special shapes of particles lead to formation of tumors? We don't have a complete answer, but, we now make the following speculation. Animal experiments and the histological investigation showed formation of tumors in the mesothelium in an overwhelming degree. Some authors have reported lung tumors following asbestos application. In our experiments subcutaneous injection of chrysotile A in one case only led to tumors in the subcutaneous connective tissue space. In addition, the storage of fibers by the lymph nodes never induced tumors of the lymphatic system. These observations suggest that tissues have different vulnerabilities to fibrous dusts. Epithelial and epithelium-like cells obviously conflict with fibrous dusts in an intensive degree; this intensive contact can be well observed in cell cultures. Beck and Bruch *(1)* demonstrated by electron microscopy a partial invagination of long asbestos and glass fibers by fibroblasts (L-cells) of mice. Although these cells can not phagocytose the fibers completely they remain dense around the foreign bodies. Such a relation between fiber and cells could support a chronically enhanced reproduction of cells within quickly regenerating epithelial and mesothelial cells. For decades it has been known that in epithelial organs like the liver and the respiratory tract chronically enhanced regeneration can change to abnormal

Table 3. Tumor rate after intraperitoneal injection of fibrous and granular dusts.

Dust	Doses (i.p.), mg	Time required to produce first tumor in animals of group, days	Tumor rate, %
Chrysotile A	6	343	67.5
Chrysotile A	25	276	65
Chrysotile A	4 × 25	270	37.5
Chrysotile A (milled)	4 × 25	400	30
Glass fibers	4 × 25	197	57.5
Nemalite	4 × 25	249	62.5
Palygorscite	3 × 25	257	65
Gypsum	4 × 25	546	5
Pectolite	4 × 25	569	2.5
Sanidine	4 × 25	743	2.5
Talcum	4 × 25	587	2.5
Actinolite	4 × 25	—	—
Biotite	4 × 25	—	—
Hematite (precipitated)	4 × 25	—	—
Hematite (mineral)	4 × 25	—	—
NaCl (control)	4 × 2 ml	—	—

regeneration and finally the tumorous cell proliferations.

We would offer for discussion the question of whether the reported reaction of mesothelial cells to fibrous particles could support the old irritation theory in carcinogenesis.

Finally, let us emphasize that the fibrosis induced by the fibrous dusts cannot be regarded as starting point of the malignant degeneration. Even ground chrysotile A induced tumors in 30–40% of the rats; those animals of this group that died without any tumor showed only slight degrees of adhesion.

The Problems of Dose–Response Correlation

The carcinogenesis depends on the shape factor of the dusts. It would be ideal for animal experiments if the fibers were of identical diameter and of identical length within one specimen. Then the dosage could be determined by the amount of fibers instead of weight. This has so far been impossible to achieve, however. The question of minimal and maximal length and diameter of the fibers necessary for a carcinogenic effect can still not be answered completely. In contrast to Wagner, Berry, and Timbrell *(2)*, we are inclined to believe that even short fibers less than 10 μm in length, can induce tumors. The milled chrysotile A contained only few fibers exceeding 10 μm in length, and 99.8% of the fibers were shorter than 5 μm. We also do not believe that the cancerogenic effect can be limited to fibers with a diameter less than 0.5 μm. Since 2 mg of fibers with a diameter of 0.5 μm and a length of 20 μm induced tumors in rats, 200 mg of fibers with a diameter of 5 μm would have to be injected to reach the same amount of fibers. We have not yet determined whether such a dosage can be survived by the animals long enough. We suppose that the maximum diameter of an effective fiber is limited by the length of the fiber that still can induce damage to the membrane of a cell. This size may range between 1 and 3 μm.

We believe that glass fibers are a better test dust for further investigations than asbestos, as glass fiber specimens with nearly identical fiber diameters can be better obtained.

To reach a better relation of the dosage and the amount of fibers we have developed a nomogram. Following measurement of sufficient fibers diameters and lengths, the number can be estimated by the nomogram.

Correlation of Experimental Results with Human Morbidity

Since glass fibers can induce tumors like those caused by asbestos fibers we would recommend precaution for all industrial plants in which there are high concentrations of fibers measuring less than 3 μm in diameter. To date, no tumors have been found in workers of the glass fiber industry, but, according to reports of the industry, thinner glass fibers (less than 5 μm in diameter) have only been in production for the last 10 years in Germany. Since the time of tumor manifestations is 20–40 years in asbestos workers, there is no epidemiological proof possible before 1985 that these experimental results apply to human morbidity.

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