PLAINTIFF'S EXHIBIT

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ANIMAL EXPERIMENTS ON BIOLOGICAL EFFECTS OF MINERAL FIBRES

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Which fibres lead to disease in human beings? The answer to this question is of central importance in the prevention of fibre-related disease. In recent years, research has concentrated on the aspect of which fibres induce tumours; this was prompted by the following observations:

(1) an increase in asbestos-induced tumours in humans;

(2) results from animal experiments that show that fibres other than asbestos can also produce tumours; and

(3) the increasing realization that fibrosis is not a necessary prerequisite for an asbestos-induced tumour.

Available results convince us that animal experiments can make an important contribution to solving the problem of defining a carcinogenic fibre. If this were not the case, then experiments using animals could not be justified.

The papers in this section are summarized under the following five subject headings:

(1) susceptibility of different animal species,

(2) behaviour of fibres in the lung,

(3) behaviour of fibres in the intestinal tract,

(4) influence of nonfibrous substances on the carcinogenicity of fibres and

(5) different biological effects of different fibres.

1. SUSCEPTIBILITY OF DIFFERENT ANIMAL SPECIES

The rat is the laboratory animal most commonly used for testing the biological effects of asbestos and other mineral fibres. The wealth of results published in recent years by many authors cannot all be cited here. However, the induction of tumours by intrapleural or intraperitoneal application in rats has been sharply criticized on the grounds that such results are irrelevant for man (Gross, 1974; Valentin, 1977). It has been argued that not only would this unphysiological route of administration restrict the possibility of extrapolation, but, moreover, that rats would react in qualitative terms differently from humans. Some years ago, however, mesotheliomas were also observed in mice (Davis, 1976; Pott et al., 1976) and Syrian golden hamsters (Smith, 1974) after intrapleural or intraperitoneal injection of fibres, and those results have since been confirmed in other experiments.

Smith et al.[1] induced tumours in golden hamsters by intrapleural injection of chrysotile and tremolite as well as glass fibres. Pylev[2] obtained numerous malignant and benign lung tumours in golden hamsters after intratracheal instillation of chrysotile or amphibole fibres. Using golden hamsters for inhalation studies with chrysotile, Wehner[3] found no malignant lung tumours but lung adenomas. Pott et al.[4] observed mesotheliomas in European hamsters after intraperitoneal injection of crocidolite and in rabbits after intraperitoneal injection of chrysotile.

Monkeys are also used for testing fibres. Pylev[2] treated rhesus monkeys with chrysotile intratracheally and intrapleurally and found asbestosis and pretumourous lesions in seven monkeys that died before preparation of his report.

These results demonstrate that not only did rats develop tumours after treatment with fibrous dusts in several studies but that in other animal species the latent period is for the most part longer and tumour incidence substantially less. It can be concluded that the serosa of several animal species react qualitatively in the same way to the stimulus of fibrous dusts, with the development of a tumour; the difference with regard to rats exists in relation to the quantity of tumours obtained. One reason may be the reduced ability of the rat to form asbestos bodies.

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[1] See . 357

[2] See . [illegible]

[3] See . [illegible]

[4] See . [illegible]

There appears to be a difference in susceptibility between rat strains, but it is not basic. Young rats showed a stronger and faster reaction after intraperitoneal injection of glass fibres than older rats (Pott et al.[1]).

2. BEHAVIOUR OF FIBRES IN THE LUNG

Morgan[2] showed that after exposure of rats to anthophyllite, fibres less than 5 µm in length are cleared from the lungs more efficiently than longer ones. Other experiments by Morgan[2] dealt with the formation of coated fibres in rats, guinea-pigs and humans. He concluded that the longer and thicker a fibre, the greater is the probability of body formation.

Wehner[3] determined the deposition, translocation and clearance of inhaled neutron-activated talc. The biological half-life of the talc deposited in the alveoli was seven to ten days. No translocation of talc to liver, kidneys, ovaries or other parts of the body was found. In contrast, the half-times of alveolar clearance of chrysotile and amphibole asbestos ranged from 60–90 days in a study by Morgan et al. (1977).

Other important aspects of the behaviour of fibres in the body are their translocation or migration and their solubility. A fibre that does not reach the pleura or peritoneum cannot induce a mesothelioma. Fibres that are dissolved soon after arriving in the serosa possess only a weak carcinogenicity for that tissue by virtue of their fibrous character.

Crapo et al.[4] found fibres in alveolar macrophages one day after the start of a seven-hour inhalation of chrysotile. Five days after the start of daily seven-hour exposures, fibres were observed within the alveolar cells, in the subepithelial basement membranes and in interstitial cells. The Mg:Si ratio of chrysotile in a control sample differed from that of chrysotile in interstitial connective tissue and in alveolar spaces after three months' exposure: the values were about 0.7, 0.6 and 0.3, respectively.

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[1] See p. 337

[2] See p. 329

[3] See p. 373

[4] See p. 273

In 1974, Gross (1976) emphasized that glass fibres could not be carcinogenic after inhalation because - in contrast to asbestos fibres - they would not migrate from the air spaces to the pleura. It has now been shown that after intratracheal instillation in European hamsters, glass fibres thinner than 0.4 μm are present in the diaphragm and spleen in the same range as crocidolite (Pott et al.[1]). A similar study should be done by inhalation.

3. BEHAVIOUR OF FIBRES IN THE INTESTINAL TRACT

The rate at which ingested fibres penetrate the gastrointestinal tract was studied in rats by Masse et al.[2] Fibres were found in the abdominal lymph from two to 24 hours after feeding, and 50% of the total absorbed amount was found in the lymph within the first six hours. The daily maximum recovery rate in the abdominal lymph amounted to 2.1×10^{-4}; in some rats no fibres were found. Large fibres were most numerous in the lymph. Initial experiments with attapulgite fibres showed negative results, but this important finding must be confirmed.

The penetration rate of fibres through the gastrointestinal wall can only be a measure of the internal body burden of fibres. The possible effect of fibres on the cells of the bowel wall is another problem. After administration of drinking-water containing 1300 million UICC amosite fibres/l, Smith et al.[3] found two early squamous-cell carcinomas of the forestomach, one peritoneal mesothelioma and one lung carcinoma among 180 golden hamsters; however, these tumours cannot be definitely attributed to the treatment.

In experiments in which 42 rats were fed asbestos filters, Gibel et al. (1976) observed a total of 12 malignant tumours in lung, liver, kidney and reticulum-cell tissue, and two liver-cell carcinomas in controls; however, in the publication the number of tumour-bearing rats was not clearly specified.

4. INFLUENCE OF NONFIBROUS SUBSTANCES ON THE CARCINOGENICITY OF FIBRES

Wyley[4] found no influence on pleural carcinogenesis of benzo[a]-pyrene adsorbed on or added to asbestos dust, confirming a result obtained in the intraperitoneal test (Pott et al., 1972). In contrast

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[1] See p. 337

[2] See p. 321

[3] See p. [illegible]

[4] See p. [illegible]

to these findings, benzo[a]pyrene stimulated the carcinogenic effect of asbestos in the lung after intratracheal instillation (Pylev & Shabad, 1973; Smith et al., 1970). A parallel can be found in humans: smoking increases the incidence of lung carcinoma but has no influence on the rate of mesothelioma.

According to the results of Küng-Vösamäe & Vinkmann[1], there is a syncarcinogenic effect of intratracheal instillation of chrysotile and oral or subcutaneous application of *N*-nitrosodiethylamine in the lungs of golden hamsters. However, the total percentage of tumours in the respiratory tract, including the lung, was slightly higher after administration of *N*-nitrosodiethylamine alone than after administration of the combination of carcinogens.

No syncarcinogenicity of chrysotile inhalation and cigarette smoke was found by Wehner et al. (1975) in golden hamsters. After 15 months of exposure to asbestos-cement, a dose-response relationship for pulmonary fibrosis was seen (Wehner et al., 1978). The authors state that they observed neither primary carcinomas of the respiratory tract or lung nor mesotheliomas. However, an animal experiment that was interrupted after 15 months with no evidence of tumours cannot be called a test of carcinogenicity.

A possible synergistic effect of chrysotile with a physical carcinogen was studied by Lafuma et al.[2]. Preliminary results after intrapleural or oral administration of chrysotile in addition to radiation in rats give a slight indication of syncarcinogenicity.

5. DIFFERENT BIOLOGICAL EFFECTS OF DIFFERENT FIBRES

This subject gives rise to a great many problems and controversies with regard to interpretation.

Davis et al.[3] carried out inhalation studies in rats to compare the effects of UICC amosite and UICC chrysotile with amosite and chrysotile samples collected from the factory environment. In earlier experiments, these workers found that long asbestos fibres are more dangerous than short ones (Davis et al., 1978), leading them to the conclusion that chrysotile might be more carcinogenic to rats than amosite, regardless of fibre length; consequently, neither a single mass standard nor the present fibre-number standards would be satisfactory. However, they indicate that it was not possible to study the importance of very thin fibres.

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[1] See p. 305

[2] See p. 311

[3] See p. 265

Crapo et al.[1] used morphometric techniques to quantitate structural changes occurring in rats after inhalation of two chrysotile samples which differed in the number of long fibres present; however, fibre size distribution was not specified exactly. Studies on lung physiology were also done: the results suggest that longer and shorter fibres cause lung injury but that long fibres have greater toxicity.

Johnson & Wagner[2] and Wagner et al.[3] investigated the effects of chrysotile and of four types of man-made mineral fibres after long-term inhalation. Preliminary findings suggest that chrysotile was the most active; similar reactions occurred with all the man-made mineral fibres, except that the noncoated glass wool was more reactive than the coated variety. However, considerably more fibres were present in the respirable fraction of chrysotile than in that of man-made mineral fibres. In another model, relatively low tumour rates occurred after intrapleural injection of these five fibre types. The highest value, 12.5%, was found in the chrysotile group, while slag wool induced no tumours. These differences can be explained by the different ranges of fibre diameters in the samples.

Intrapleural experiments in hamsters with glass fibres of different lengths and diameters were carried out by Smith et al.[4] Tumours developed only in the groups treated with samples that had a substantial percentage of fibres longer than 20 µm; however, the highest tumour incidence obtained after injection of the longest and thinnest fibres was only 15%. These results cannot therefore prove the harmlessness of fibres shorter than 20 µm.

Until now, the most detailed statement on the carcinogenicity of different size categories was made by Stanton and his group (Stanton & Layard, 1978; Stanton et al., 1977) on the basis of results obtained after intrapleural application of 37 man-made mineral fibres with different particle size distributions. The particle size distributions of the samples were graded into 34 size categories and correlated with the tumour rates. The optimum correlation was obtained with fibres less than 0.25 µm in diameter and more than 8 µm in length. The authors pointed out that the absence of a correlation does not preclude a low level of tumour response outside these ranges.

Pylev[1] tested three chrysotile and four amphibole samples by intrapleural injection in rats and two chrysotiles and two amphiboles by intratracheal instillation in golden hamsters. The lengths of all seven samples are described as approximately similar: more than 90% of the particles were shorter than 5 µm. The tumour incidences ranged between 41 and 77%, except in the two groups treated with synthetic chrysotile which had only a few tumours.

In an experiment by Lafuma et al.[2], the carcinogenicity of chrysotile was decreased after leaching. The same result was obtained by Morgan et al. (1977) and by Davis et al.[3] after heating chrysotile to 850 °C.

Last but not least, Wagner et al.[4] carried out inhalation and injection studies with three samples of Canadian chrysotile in rats, following on preceding experiments by this group (Wagner et al., 1973, 1974). The special feature of the results is that inhalation and injection of the three chrysotile samples caused a different sequence of carcinogenicity in the two tests: UICC chrysotile showed the highest carcinogenicity of the three samples after inhalation and the lowest after intrapleural injection. This difference seems to worry the authors more than it did the reviewer. It must be remembered that the target tissue in one test is the bronchial epithelium, where carcinomas or adenomas are produced; in the other test, it is the serosa, where mesotheliomas are produced. However, it might be possible to explain the results only on the basis of the effective number of fibres; we do not know enough about the number of fibres that reaches the bronchial cells or the serosal cells from inhalation of dust which contains the same mass of fibres but different particle sizes.

CONCLUSIONS

With these considerations I finally come to some general remarks. It seems to be well established that a long, thin fibre has more carcinogenic and fibrogenic potency than a short and/or thick fibre, although it is not proven that fibres shorter than 5 µm cannot induce tumours or fibrosis. From the physiological point of view, there cannot be a large difference between a fibre 4.9 µm in length and a fibre 5.1 µm in length with regard to their carcinogenicity. Figure 1 (Pott, 1978) illustrates a hypothesis concerning the carcinogenic potency of

[1] See p. 343

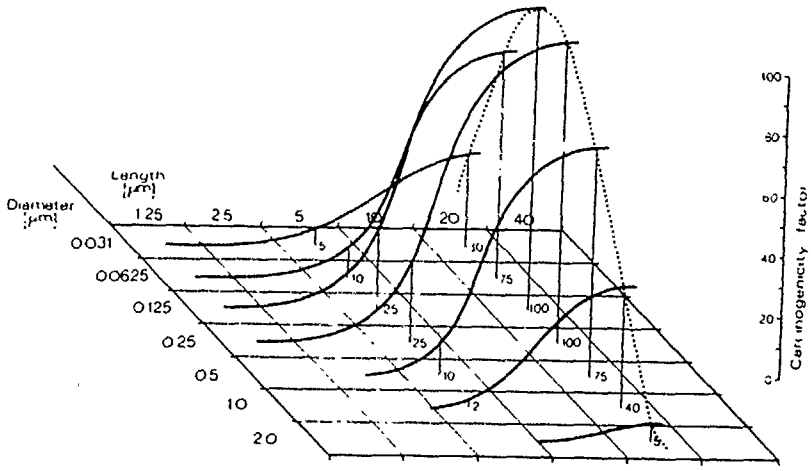
[2] See p. 311

See p. 285

[4] See p. 363

FIG. 1. HYPOTHESIS CONCERNING THE CARCINOGENIC POTENCY OF A FIBRE AS A FUNCTION OF ITS SIZE USING DATA ON 'CARCINOGENICITY FACTORS'

This three-dimensional model requires that the fibre size of a sample be divided into numerous categories; the size categories include three parameters: length, diameter and the length:diameter ratio.



fibre as a function of its size, using data on carcinogenicity factors for size categories, which have to be determined experimentally. On the basis of this hypothesis, the carcinogenic potency of short fibres may be weak, but many short fibres may induce a tumour as easily as a few long fibres. The more recent results of Stanton - as far as I know - are compatible with this working hypothesis.

At the Lyon conference on asbestos in 1972, Wagner & Berry (1973) called it a disappointment that too few experiments were carried out with the UICC reference samples of asbestos dusts. The situation has not changed since. The good idea of producing and recommending five standard samples for experimental studies could not lead to results of the desired extent because of the great variation in the chemical and physical properties of the minerals and emitted particles that exist in the environment. Many experimentalists use dusts that they prepare themselves; but these dusts are available only in small amounts and are hardly reproducible; above all, their chemical and physical properties are often insufficiently characterized. It is therefore very difficult to prove the significance of fibre sizes or of other properties which exist independently of length, diameter and solubility. It is nearly impossible to get two fibre samples which differ only in one property. Each treatment of a fibre sample - especially milling - changes several properties simultaneously (Langer et al., 1978).

These difficulties may be trivialities for many of you. However, as an advocate of the experimentalists I appeal to the mineralogists and aerosol physicists with the urgent request to help us in this serious situation: we need, instead of five, at least 50 well-defined standard samples of asbestos and man-made mineral fibres. That may seem to be very presumptuous and expensive. However, in the present state we are experimenting with several hundred dusts and have great difficulties in comparing and interpreting the results. That state cannot be less expensive.

SUMMARY

The papers presented in this session are summarized. Although asbestos fibres produce tumours in a number of animal species tested, rats appear to be the most susceptible, in terms of latent period and numbers of tumours produced. The deposition, translocation and clearance of different types of fibres in the lung have been investigated in a number of experiments, and it has been shown that many of them migrate more readily than was previously thought; their penetration into the gut was the object of further investigation. The syncarcinogenicity with asbestos of various substances, such as benzo[*a*]pyrene, *N*-nitrosodiethylamine, cigarette smoke or radiation, is described. Experiments on the different carcinogenicities of different fibres are summarized; although it is pointed out that there is much controversy in this area. A hypothesis is presented whereby the carcinogenic potency of a fibre is dependent on various size parameters, based on length, diameter and length:diameter ratio. On the basis of this hypothesis, the carcinogenic potency of short fibres may be weak, but many short fibres may induce a tumour as easily as a few long fibres. Finally, a plea is made for a far greater number of well-defined standard samples of asbestos and man-made mineral fibres than exists at present, since there are currently great difficulties in comparing and interpreting results.

RESUME

L'auteur résume les communications présentées. Si les fibres d'amiante provoquent des tumeurs dans plusieurs espèces animales testées, le rat semble être la plus sensible, quant à la période de latence et au nombre de tumeurs induites. Le dépôt, le déplacement et l'épuration de différentes variétés de fibres dans le poumon ont été étudiés lors de plusieurs expériences, et l'on a montré que beaucoup d'entre elles migrent plus aisément qu'on ne le pensait; leur pénétration dans l'intestin a fait l'objet de nouvelles investigations. L'auteur décrit la cocancérogénicité avec l'amiante de diverses substances, comme le benzo[a]pyrène, la N-nitrosodiéthylamine, la fumée de cigarette, ou bien des rayonnements. Il résume les expériences effectuées sur les différents effets cancérogènes de diverses fibres; mais en soulignant que la question est très controversée. Est présentée une hypothèse selon laquelle le pouvoir cancérogène d'une fibre dépend de divers paramètres de taille: longueur, diamètre et rapport longueur:diamètre. Selon cette hypothèse, le pouvoir cancérogène des fibres courtes peut être faible, mais beaucoup de fibres courtes peuvent provoquer une tumeur aussi aisément que quelques fibres longues. Enfin, l'auteur plaide en faveur d'un bien plus grand nombre d'échantillons étalons bien définis d'amiante et de fibres minérales artificielles qu'il n'en existe à l'heure actuelle, étant donné les grandes difficultés qu'on éprouve présentement à comparer et à interpréter des résultats.

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