

Information obtained from animal experiments

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Evidence was presented at the New York meeting in 1964 that mesotheliomas could be produced in hamsters and rats by intrapleural inoculation of asbestos dusts (Smith *et al.*, 1965; Wagner, 1965). This opened up the possibility of using animal experimentation as a means of investigating the etiology of mesotheliomas caused by asbestos. In this paper, the results of experiments published or made available to us since then are summarised. For convenience the experiments will be divided into four categories according to the route by which the asbestos was administered: first, intrapleural; secondly, intraperitoneal; thirdly, intratracheal; and fourthly, inhalation experiments. The different routes are considered by Wagner & Berry² at this meeting, and fuller details of the methodology are given in the references below.

INTRAPLEURAL EXPERIMENTS

All types of asbestos have produced mesotheliomas in rats. Wagner (1972) reported an experiment in which inoculations with all five UICC standard reference samples (Timbrell *et al.*, 1968) produced mesotheliomas: with a dose of 20 mg these tumours occurred in 61% of animals for crocidolite, 36% for amosite, 34% for anthophyllite, 30% for Canadian chrysotile and 19% for Rhodesian chrysotile. Stanton & Wrench (1972), with a dose of 40 mg of asbestos dust on gelatine-coated fibre-glass pledgets, found that three of the UICC samples, crocidolite, amosite and Rhodesian chrysotile, all produced mesotheliomas in about 60% of rats.

In a comparison of different doses, using crocidolite and chrysotile (not the UICC samples), the risk of developing a mesothelioma at a given age was found to be proportional to the dose (Wagner *et al.*, 1970). The effect of dose was also demonstrated by Stanton & Wrench (1972) for UICC crocidolite, as well as for another sample of crocidolite.

Mesotheliomas have also been produced by other workers (Donna, 1970; Reeves *et al.*, 1971; Smith *et al.*, 1965) in rats, hamsters and rabbits, including 23 out of 50 rats inoculated with 25 mg of UICC Rhodesian chrysotile (Wheldon, 1972³).

The suggestion that natural oils and waxes (Harington, 1962) and, subsequently, contaminating oils both from the preparation of the fibre (Harington & Roe, 1965; Roe *et al.*, 1966) and from plastic storage bags (Commins & Gibbs, 1969) played a part in the development of the tumours, has been refuted. The sample of crocidolite from which the oils were removed by Harington gave very similar results to the untreated sample (Wagner & Berry, 1969). Also, all five UICC samples from which the oils were removed by Commins have been compared with identical untreated materials, and the oil-extracted samples produced 58 mesotheliomas compared with 56 with the untreated samples (Wagner, 1972).

Another suggested explanation for the carcinogenicity of asbestos was that the presence of the trace metals might be relevant (Harington & Roe, 1965). This received support from Wagner *et al.*, (1970), who reported that for samples of chrysotile from seven different Canadian mines there was a significant correlation between the production of mesotheliomas and their chromium content. However, Wagner (1972) discounted this finding, since the published chromium content of one of the

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² See p. 85 of this publication.

³ Personal communication.

samples had subsequently been amended and the correlation was no longer present.

Wagner *et al.* (1970) suggested that the fineness of the grinding of the samples was of importance. Evidence of this was found by Wagner (1972) from an experiment in which UICC Canadian chrysotile had been compared with each of the eight separate samples which had been combined to form the UICC sample (Timbrell & Rendall, 1971). All of the separate samples produced more mesotheliomas than did the UICC sample and had been ground more finely before experimental use. However, a different mill had been used, so that factors other than particle size could not be ruled out. Of the eight separate samples, one was from a mine in Western Canada whilst the others were from a relatively small area of Eastern Canada. The sample from Western Canada was the least carcinogenic in each of two experiments, but it could not be separated from the other samples on the basis of its size distribution. However, the sample of chrysotile which unexpectedly produced mesotheliomas in as many as 66% of rats (Wagner & Berry, 1969) was a super-fine sample, produced by sedimentation separation from grade 7 (the most fully milled of all commercial products).

The effect of grinding was also investigated by Stanton & Wrench (1972), who found that UICC crocidolite when partially pulverised produced fewer mesotheliomas than did the standard sample. They postulated that submicroscopic fibres, i.e., smaller than about $1.25 \times 3.75 \mu\text{m}$, could be discounted. The apparent disagreement on the significance of fibre size is discussed in the following two papers (Stanton¹; Timbrell²).

INTRA-PERITONEAL EXPERIMENTS

Peritoneal mesotheliomas have been produced by Reeves *et al.* (1971) in Charles River caesarean-derived rats after intraperitoneal inoculation with crocidolite and chrysotile, but not with amosite. They have also been produced by Davis (1972³) in 17 out of 25 rats and 23 out of 50 mice injected with crocidolite.

¹ See p. 289 of this publication.

² See p. 295 of this publication.

³ Personal communication.

INTRA-TRACHEAL INOCULATION

This method has been used to study the co-carcinogenesis of chrysotile fibre with benzo(a)pyrene by Miller *et al.* (1965) and by Vösamäe (1972). Miller and colleagues used hamsters, and Vösamäe rats. The results of both experiments demonstrate that the presence of the chrysotile has a promoting action on the carcinogenicity of the benzo(a)pyrene.

INHALATION EXPERIMENTS

Gross *et al.* (1967) reported the production of carcinomas of the lungs of rats exposed to chrysotile dust. Of 72 rats which survived 16 months' exposure with a mean concentration of 86 mg/m^3 for 30 hours a week, 24 animals developed carcinomas and there was one mesothelioma. It was considered that trace metals from the worn hammer of the mill used to produce the respirable fibre could be a factor in the causation of these tumours.

Reeves *et al.* (1971) found squamous carcinomas in 2 rats out of 31 which survived two years of crocidolite exposure with a concentration of 49 mg/m^3 for 16 hours per week. Five of those exposed to chrysotile developed pulmonary adenomatosis, but there were no malignant tumours among those exposed to either chrysotile or amosite.

Wagner (1972) reported an experiment in which rats were exposed to a concentration of 12 mg/m^3 of respirable dust of each of the five UICC samples. There were two lengths of exposure, 1 day (7 hours) and 3 months (400 hours). The amount of chrysotile retained in the lungs at the end of the longer period of exposure was only one sixth that found with the three amphibole samples. Three mesotheliomas were observed, and two of these occurred after the shorter exposure, one with amosite and one with crocidolite. The third was a peritoneal tumour occurring after the longer exposure to crocidolite; a squamous-celled carcinoma of the lung also occurred in this group. An excess of lung adenomas over non-exposed controls was observed after the longer exposure for all dusts except anthophyllite. No explanation could be offered for the surprising finding that two mesotheliomas occurred after the shorter exposure compared with only one after the fifty times more severe exposure. The probability of such an extreme result occurring by chance is only about 2 in 1000.

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The experiment has now been supplemented by one with exposures of up to 2 years. In this study all types of fibre produced asbestosis, and the fibrosis was progressive after exposure was ended. Also, all samples, including anthophyllite, produced an excess of lung tumours. These tumours varied from adenomas to adeno-carcinomas; and squamous cancers and the occasional mesothelioma were also produced. Surprisingly, a few of the mesotheliomas found occurred with Canadian chrysotile.

DISCUSSION

Most of the experimental work has involved application of asbestos either intrapleurally or by inhalation. The former may be rightly criticised as being unrealistic, but it has nevertheless an important part to play. In an inhalation experiment two factors must be borne in mind: firstly, the dust must penetrate through the airways and alveoli, and this may differ with different samples of asbestos; and secondly, that the dust, given that it has reached the pleura, exercises a "biological activity". In contrast, in inoculation experiments only the "biological activity" needs to be considered, and this makes these experiments the more readily interpretable. The two types of experiment supplement one another, intrapleural application being much more suitable for investigating questions such as whether extraction of the oils alters the carcinogenicity of a sample. In addition, the ease of production of mesotheliomas by the intrapleural route provides a useful experimental model.

From the results of intrapleural studies it seems unlikely that the presence of oils and waxes, whether natural or acquired, plays a significant part in the development of mesotheliomas. The possibility that trace elements contaminating the fibre play a significant role as carcinogenic factors also receives no support from the experimental evidence. The fact that the different types of asbestos, with very different chemical compositions, can all produce mesotheliomas, also makes it unlikely that there would be a chemical explanation for the carcinogenicity of

asbestos. The contamination of the fibre may have had some significance in the inhalation experiments of Gross and his co-workers (1967), but not in the larger study with standard reference samples.

The most interesting pointer obtained from these experimental results is that development of mesotheliomas is associated with the presence of fine fibrous material within the pleural cavity. This theme is elaborated in the following two papers (Stanton¹; Timbrell²).

If physical characteristics are the important factors as far as carcinogenesis is concerned, then one might expect that other fibrous material would produce mesotheliomas. In fact, other materials have produced mesotheliomas after intrapleural application. It should be noted, however, that the tumours produced by silica and reported as being similar to mesotheliomas by Wagner (1965) have now been shown to be lymphomas and not mesotheliomas (Wagner & Wagner, 1972). Stanton & Wrench (1972) reported the occurrence of mesotheliomas with silicon dioxide, glass-wool and fibre-glass, the finer samples of fibre-glass producing the highest incidence (18%). Occasional tumours were also produced by Webster (1965) with carbon black, and in our own experiments by synthetic aluminium silicate fibre, barium sulphate, glass-powder and aluminium oxide.

Experiments designed to investigate in more detail the role of the size and shape of asbestos and other fibres would seem to be a profitable way of advancing current knowledge.

Additionally, intrapleural methods have provided a source of tumours, histologically indistinguishable from those seen in man, which can be used for immunological investigations and to study the response to therapy.

Inhalation experiments have provided evidence for estimating the dose required to produce a low incidence of tumours. This opens up the possibility of using inhalation to investigate the effects of combined exposure to asbestos with other agents, such as smoking.

¹ See p. 289 of this publication.

² See p. 295 of this publication.

SUMMARY

Experiments in which animals have been exposed to asbestos are reviewed, mainly from the carcinogenic viewpoint. Most work has involved applying the dose either

intrapleurally or by inhalation; and the animal used most has been the rat. After intrapleural administration a high proportion of animals usually developed meso-

theliomas, and results show that the carcinogenesis of asbestos is unlikely to be due to the oils and waxes present in asbestos or to trace elements. There are indications that fibre size and shape are important, and it is suggested that further work on this aspect might be

profitable. Mesotheliomas have been produced with fibre-glass and other non-asbestos materials. Mesotheliomas have occurred only occasionally after experimental inhalation, but adenomas and adeno- and squamous carcinomas occurred more frequently.

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