In: Pneumoconiosis. Proceedings of the International Conference, Johannesburg. 1969.

MESOTHELIOMAS IN RATS

FOLLOWING THE INTRA-PLEURAL INOCULATION OF ASBESTOS

J. C. WAGNER, G. BERRY *and* V. TIMBRELL
Pneumoconiosis Research Unit of the Medical Research Council, Penarth, Glamorgan

Epidemiological studies have suggested that mesotheliomas of the pleura and peritoneum are related to exposure to asbestos dust. Pleural mesotheliomas may be produced in rats following intra-pleural inoculation of asbestos dust and experiments in animals may help in the determination of the factors influencing the occurrence of such tumours. In this paper the results of three experiments in which asbestos was inoculated intra-pleurally into rats will be given.

EXPERIMENTS

EXPERIMENT A: COMPARISON OF TYPES OF ASBESTOS

The treatments were amosite, chrysotile, crocidolite, oil-extracted crocidolite and a saline control. There were 56 S.P.F. rats per treatment and the experiment was duplicated with a similar number of Standard rats. The dose was 20 mg. of dust per rat and the experiment was started at the end of 1962.

EXPERIMENT B: VARYING DOSE EXPERIMENT

This experiment involved only chrysotile and crocidolite each at 5 doses, 0.5, 1, 2, 4 and 8 mg. per rat with 12 rats per dose per dust. Injection was during March 1965.

EXPERIMENT C: COMPARISON OF CANADIAN CHRYSOTILES

Samples of chrysotile from seven Canadian mines were used, the same chrysotile as in experiments A and B and a saline control. The dose was 20 mg. per rat, and there were 16 rats per Canadian chrysotile, 32 for the original sample of chrysotile and 48 controls. Injection was during December 1966.

All the experiments were survival experiments, i.e. the animals were only killed if they appeared to be distressed. Experiments A and B are complete but 20% of the rats in Experiment C were still living at the end of January 1969 so that the results presented of this experiment are not final.

A necropsy examination was carried out on each animal. Of the rats inoculated with asbestos an appreciable proportion developed mesotheliomas in all the experiments but we have never found this type of tumour with the control treatments. Thus a mesothelioma can be said to be a direct result of inoculation with asbestos.

METHOD OF ANALYSIS

The treatment comparisons to be given are based on the incidence of mesotheliomas. The proportion of animals developing mesotheliomas may be used for comparison within an experiment but it has to be realized that this proportion is the result of two factors, the first being the natural mortality experienced by a group of animals whether injected with asbestos or not, and the second the increased mortality due to the risk of developing a mesothelioma. It is the latter component we are interested in and we would like to separate it from the former. With large groups of animals such as in experiment A this can be achieved by life table methods and the result of the calculations depicted by a survival curve showing the proportion of animals alive at a given time when the animals were at risk of developing a mesothelioma but the risk of natural death has been eliminated. If we knew the mathematical form of this curve we could estimate its parameters and effectively produce a smoother version of the survival curve. This would be particularly useful for experiments B and C where small groups of animals were used and it would be expected that the calculated survival curves would show large irregularities.

A model relating the induction rate of tumours with time was given by Neyman (1953) and developed by Armitage *et al.* (1954) and Pike (1966) and has been used both for analysing epidemiological and animal experiments. In our situation, if m is the age-specific death rate of animals with a mesothelioma at time t after injection the model is

$$m = ck(t-w)^{k-1} \text{ for } t \geq w$$

where c, k and w are constants. Various theoretical approaches lead to this model and it may be considered as the third extreme value distribution arising

number of cells are considered at risk of malignancy and a cancer cell is formed when the first such cell succumbs. Secondly the model would hold if a cancer cell was the end result of k successive cellular changes. With both approaches the constant c is related to the dose and w is the induction or latent period. In our analysis we will also describe the age-specific natural death rate as $\exp(a + bt)$ which gives a satisfactory fit to our control groups and has been used in epidemiological studies.

Results

Experiment A. The percentages of rats developing mesotheliomas are given in Table 1. We note that the SPF and Standard rats

Table 1: Percentage of Rats Developing Mesothelioma—Experiment A

	SPF	*Standard*
Amosite	40	31
Chrysotile	64	69
Crocidolite	59	68
Oil-extracted Crocidolite	59	64

gave similar results, that amosite gave fewest mesotheliomas and that the oil-extracted crocidolite gave similar values to the natural crocidolite. Calculation of the survival curve eliminating natural death, i.e. considering only

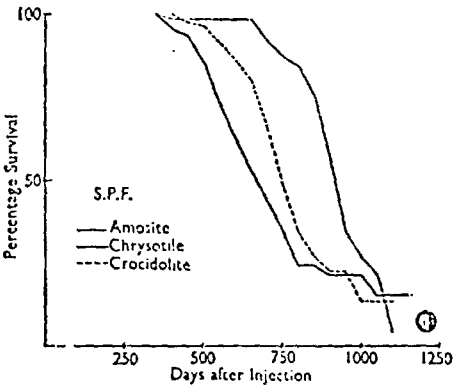

Fig. 1. Experiment A: Survival of S.P.F. rats with mesotheliomas after eliminating effect of mortality due to other causes.

mesotheliomas as causing death, gives Fig. 1 for SPF rats. For convenience of presentation the oil-extracted crocidolite has been excluded since its results were so similar to the natural crocidolite. For each dust the pattern was similar, consisting of an initial period during which no mesotheliomas were found followed by a rapid onset of cases. However, the length of the initial period, i.e. the induction period, was dependent on the dust. For Standard rats similar results were obtained but the difference between chrysotile and crocidolite was smaller.

The model given earlier has been fitted to this experiment and generally gave good fits. The constant k had values near to 3 and may be taken as 3 without any loss of precision (this value has been assumed to hold for experiments B and C also). As an

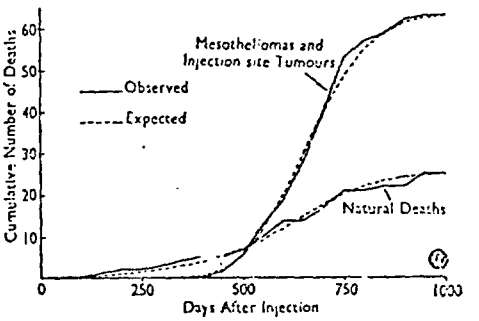

Fig. 2. Experiment A: Comparison of observed and expected distribution of deaths for crocidolite in Standard rats. The expected deaths are calculated from the model given in the text with $a = -10{\cdot}44$, $b = 0{\cdot}00586$, $c = 1{\cdot}54 \times 10^{-6}$, $k = 3$, $w = 332$.

illustration of the fit of the model, Fig. 2 shows the cumulative number of deaths with and without mesotheliomas for crocidolite in Standard rats; there is close agreement between the observed and expected distributions.

Experiment B. Averaged over doses, 36% and 19% of rats developed mesotheliomas for chrysotile and crocidolite respectively. For each dust the constant w was assumed independent of dose. This is required on theoretical grounds but with such a small experiment the data cannot be used to check this assumption very rigorously. There is a relationship between the estimate of the constant c and dose. With only 12 rats per dose the scatter is large but it has been shown that the relationship may be taken as linear, i.e. at any age the risk of developing a mesothelioma is proportional to amount of dust injected. The induction period was 100 days longer for crocidolite than chrysotile.

Fig. 3 shows the observed and expected numbers of mesotheliomas. If we extrapolate the results of this experiment to a dose of 20 mg. we find that for both dusts this would lead to a higher rate of mesotheliomas than actually occurred in experiment A, the difference being significant for chrysotile but not for crocidolite.

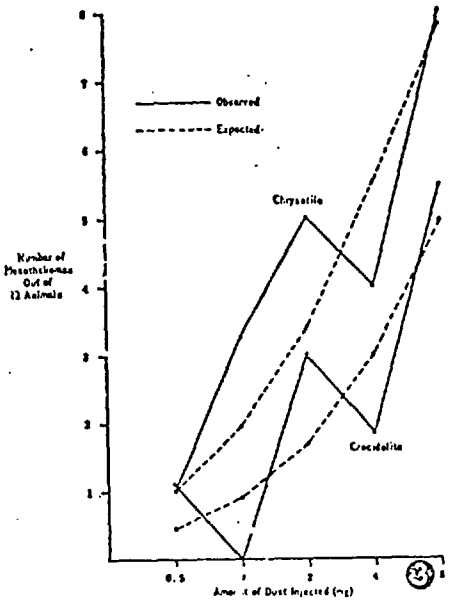

Fig. 3. Experiment B: Observed and expected numbers of mesotheliomas (out of 12 rats) on the basis that the risk of developing a mesothelioma at any age is proportional to amount injected.

Experiment C. At this stage 69% of the rats injected with the original chrysotile have developed mesotheliomas and between 19 and 56% for the 7 samples from different Canadian mills.

Again w was assumed constant for the different forms of chrysotile and in fact the best value was almost identical with that of experiment B. Comparing the treatments in terms of the parameter c, this parameter was significantly larger for the original chrysotile than the Canadian samples and the value fitted in with what would be extrapolated from experiment B.

In Table 2 are shown the values of c, and the brucite, cobalt and chromium contents of the dusts. There is correlation between cobalt and c, but mine C, which has the lowest value of c but an average cobalt content, does not fit into the same trend as the other mines. In fact the correlation is significant only if mine C is excluded. The correlation between c and chromium does not suffer from this disadvantage since mine C has a low chromium content. The relationship between c and chromium is shown in Table 3. The correlation is high (0.91) and significant ($p < 0.01$). Nickel, scandium and iron have also been looked at. Nickel gives a similar pattern to cobalt except that mine C is even more divergent from the relationship shown by the other 6 mines. The relationships of c with scandium and iron showed nothing of interest. Mine C is in western Canada, the other 6 in a relatively small area of eastern Canada.

TABLE 2: VALUES OF c AND BRUCITE CONTENT EXPERIMENT C

Mine	$c \times 10^8$	*Brucite Content*
B	9·1	20%
D	8·7	Trace
F	8·5	Trace
E	6·9	Trace to 5%
A	5·4	Trace to 5%
H	4·3	Trace
C	2·0	Absent

TABLE 3: INTRA-PLEURAL INOCULATION OF CANADIAN CHRYSOTILES
VALUES OF c (CARCINOGENICITY) AND CHEMICAL PROPERTIES

Mine	$c \times 10^8$	*Brucite Content*	*Cobalt (ppm)*	*Chromium (ppm)*
Original Sample	17·7	—	—	—
B	9·1	20%	110	780
D	8·7	Trace	78	930
F	8·5	Trace	78	730
E	6·9	Trace to 5%	57	440
A	5·4	Trace to 5%	63	520
H	4·3	Trace	43	480
C	2·0	Absent	60	120

DISCUSSION

All these experiments were started before the U.I.C.C. Reference Samples became available but the seven Canadian dusts used in experiment C came from 7 of the 8 mines from which material was supplied to form the Canadian chrysotile sample. Also, the sample of chrysotile used in all three experiments was a super-fine grade from one of these

mines (D). The difference observed between the original chrysotile and the sample from mine D in experiment C is therefore of interest and could be due to different milling procedures or since the samples were obtained several years apart due to coming from a different part of the mine. It is also of interest to note that the mesothelioma rate due to the original chrysotile in experiment B and C is greater than in experiment A. This could be due to a change in the susceptibility of the rats or a change in the dust during storage. An injection experiment was started in 1967 using the U.I.C.C. Reference Samples, repeating the separate Canadian samples and including a pure brucite treatment, this last treatment being of interest in view of the results of experiment C.

The procedure of intra-pleural inoculation is unrealistic when compared with human experience and results obtained from inhalation experiments would be more informative. Chrysotile has been shown to be a biologically active dust but epidemiological studies suggest that crocidolite is more dangerous. This may be because the spiral-like shape of chrysotile fibres inhibits their inhalation. In 1967 rats were exposed in chambers to dust clouds of the reference samples and 3 months ago another inhalation experiment was started.

Summary

Rats have been inoculated intrapleurally with samples of asbestos. With all types of asbestos an appreciable proportion of animals developed mesotheliomas. There was no difference in effect between the natural and oil-extracted forms of crocidolite. Amosite produced fewer mesotheliomas than did chrysotile and crocidolite, this being a result of a longer induction period.

When different doses were applied the risk of developing a mesothelioma at a given age could be taken as proportional to the dose.

Samples of chrysotile from seven different Canadian mines all produced mesotheliomas.

We are grateful for the help we have received from our colleagues, Mr. D. E. Munday, Mr. W. H. Roberts and Mr. J. W. Skidmore.

The trace element results were determined by Mr. A. Morgan and his co-workers.

References

Armitage, P. and Doll, R. (1954): Brit. J. Cancer, 8, 1.
Nordling, C. O. (1953): Brit. J. Cancer, 7, 68.
Pike, M. C. (1966): Biometrics, 22, 142.