WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

BIOLOGICAL EFFECTS OF ASBESTOS

Proceedings of a Working Conference held at the International Agency for Research on Cancer, Lyon, France, 2 – 6 October 1972

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IARC Scientific Publications No. 8

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER
LYON
1973

The International Agency for Research on Cancer (IARC) was established in 1965 by the World Health Assembly as an independently financed organization within the framework of the World Health Organization. The headquarters of the Agency are at Lyon, France, and it has Research Centres in Iran, Kenya and Singapore.

The Agency conducts a programme of research concentrating particularly on the epidemiology of cancer and the study of potential carcinogens in the human environment. Its field studies are supplemented by biological and chemical research carried out in the Agency's laboratories in Lyon and, through collaborative research agreements, in national research institutions in many countries. The Agency also conducts a programme for the education and training of personnel for cancer research.

The publications of the Agency are intended to contribute to the dissemination of authoritative information on different aspects of cancer research.

The authors alone are responsible for the views expressed in the signed articles in this publication.

PRINTED IN SWITZERLAND

PDF ISBN 978-92-832-2213-2

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FOREWORD

The study of the effects on man of the inhalation of asbestos dust goes back long before the International Agency for Research on Cancer came into being. The International Union Against Cancer (UICC) through its Committee on Geographical Pathology was co-ordinating an international study from 1964 within which the group working at the Medical Research Council Pneumoconiosis Unit, Penarth, UK was playing a leading part.

When the research programme of IARC got under way, asbestos was one of the environmental carcinogens that demanded our attention and we were very fortunate to have been able to conclude a research agreement with the MRC Pneumoconiosis Unit to permit the intensification of their on-going study.

The international collaborative programme involved work in many different centres and so the proposal to bring all the people together for a meeting to assess the present state of knowledge of the relation between asbestos and cancer was both timely and welcome.

We are extremely grateful therefore to Dr Gilson, Dr Wagner, Dr Timbrell and their colleagues who co-operated so strongly with us in the organisation of the meeting in Lyon. We were especially pleased that the first occasion on which we used our new building brought together so many scientists from all over the world. The meeting was most successful. I hope that this present volume will prove to be as valuable.

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INTRODUCTION

The modern asbestos industry dates from the discovery in the 1870's of large deposits of chrysotile fibre in Canada and Russia, followed by the commercial exploitation of three other types of fibre—crocidolite, amosite and anthophyllite. Previously regarded as curiosities, these fibrous silicate minerals rapidly found applications, including large-scale use in fire-proofing and reinforcement for cement in building materials. There are now numerous products which depend upon asbestos, some of which require specific types of fibre. This demand has led to a vast increase in production to the present level of more than four million tons per annum. As no satisfactory substitutes have been found for many of these uses, asbestos is likely to remain an essential material for years to come.

By the 1950's it was accepted that the inhalation of asbestos dust could lead to pulmonary fibrosis and carcinoma of the lung, and more recently that an association exists between exposure to asbestos dust and the development of diffuse mesotheliomas of the pleura and peritoneum. The mesotheliomas appeared to occur after a long lapse period, forty years, following in some cases only slight exposure to asbestos dust. Anxiety that sufficient exposure to produce mesotheliomas might occur in non-industrial situations indicated a need for an urgent assessment of the situation. It became essential to establish which type, or types, of asbestos were responsible for the development of the conditions, and to clarify the evidence through epidemiological and experimental studies. It might then be possible to suggest methods for the prevention of the diseases.

In 1963, following correspondence with Dr T. Mancuso, and with advice and encouragement from Dr H. L. Stewart, a small planning group was organised to consider the feasibility of an international approach to the investigation of the cancers associated with exposure to asbestos dust. This committee reported to Dr J. Higginson, who was then Chairman of the Geographical section of the UICC. It was planned that a working party should be convened. At this stage a request was received from Dr I. J. Selikoff to take part in a New York Academy of Sciences Conference on "The Biological Effects of Asbestos" (New York, October, 1964). The working group met after this Conference, summarised the available knowledge on "asbestos and cancer" and made recommendations for further investigations. A sub-committee was formed to co-ordinate the implementation of these proposals.

In order to widen international aspects of the study a second conference was held in Dresden in 1968. This was a most successful meeting at which valuable information was presented and further international studies were planned; the proceedings of this conference have been published recently[1].

With the establishment of the International Agency for Research on Cancer, it was considered that the asbestos problem, which was now being investigated by epidemiologists, morbid anatomists, experimental pathologists, physicists and chemists, was exactly the type of study to be integrated into the Agency's programme of environ-

[1] Holstein & Anspach, eds. (1973) *Internationale Konferenz über die biologischen Wirkungen des Asbestes, Dresden, 1968*

mental carcinogenesis. Accordingly, a research agreement was concluded with the Agency in 1968, and in 1970 the project was expanded with the Agency's support. The following year it was decided that a further meeting of the working group was appropriate to review the progress that had been made in implementing the 1964 recommendations and to collate the information now available on these cancers.

As it was considered that the situation should be reviewed on a wide basis, the working party was requested to "assess the biological effects of asbestos". Authorities on various aspects of the problem were invited to submit papers which were circulated beforehand and presented for discussion; these were summarised by rapporteurs.

After the conference, an advisory committee to Dr J. Higginson, the Director of the IARC, met to prepare a progress report and to make recommendations for further work. This study has completed what we consider to have been a successful eight-year phase. The proceedings of the conference and the recommendations of the advisory committee to the IARC have now been prepared as a publication, which we trust will provide an authoritative international review of the asbestos cancer problem and show where further research is most needed.

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SCIENTIFIC PAPERS

PRESENTATION OF REPORTS ON PROGRESS MADE ON THE RECOMMENDATIONS OF THE UICC WORKING GROUP ON ASBESTOS AND CANCER, 1964

Chairman — J. Higginson

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Progress in epidemiology

J. C. GILSON [1]

The UICC Working Group in 1964 divided its recommendations on epidemiology into two groups —those concerned with methods and those outlining projects which were thought most urgent and of special importance in prevention. Table 1 gives the recommendations.

METHODS

General

Stress was placed on the need for a very complete occupational history and the recording of other relevant factors, including smoking. Papers published since 1964 show a wide appreciation of the diversity of occupations in which asbestos exposure may occur. It has become clear that a higher proportion of workers than was previously thought may have had some exposure to asbestos (McEwen *et al.*, 1970; McDonald *et al.*, 1970; Oldham, 1972).

Inter-relation of cigarette smoking and exposure to asbestos on the incidence of related cancers is now much clearer. Newhouse [2] and Hammond & Selikoff [3] bring this information up to date. The findings have an important bearing on prevention. The relation between past exposure to asbestos and deaths from "all causes" and to individual diseases is now much better documented as a result of a number of new surveys. Knowledge in the whole field is presented and discussed in the sessions concerned with asbestosis and cancer in relation to type of fibre, dose, occupation and duration of exposure at this Conference [4]. In a majority of the recent surveys comparison groups, usually derived from national or regional mortality data, but sometimes specially selected groups (Meurman *et al.*[5]), have been included in the analyses. Not much progress has yet been reported of studies of families or households, except in isolated case reports.

Clinical criteria

A majority of the special surveys have followed the recommendations by using standardised questionnaires often based on the Medical Research Council (1960) questionnaire concerning respiratory symptoms, with additional and more detailed information about occupational history. Questions specifically related to cardiac disease have been less generally recorded.

Clubbing of the fingers has been shown to be unreliably recorded except when gross; however, efforts to measure quantitatively the change of shape have met with some success. Basal rales have been shown to be a useful and sometimes early sign. They are also related to the intensity of exposure to asbestos dust (Harries [6]). An endeavour to separate the effects of bronchitis from those caused by asbestos has been made by Thomson and his colleagues (Regan *et al.*, 1971), but in general this topic has not received much detailed study.

Classification of chest radiographs

It is encouraging to record that the first sentence of the Working Party's report on this subject, "There is no international or national standardised classification of the radiological appearances of asbestosis", is no longer true. The co-operation engendered by the New York Academy of Sciences Symposium on the "Biological Effects of Asbestos" and the

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[2] See p. 203 of this publication.

[3] See p. 312 of this publication.

[4] See pp. 155–227 of this publication.

[5] See p. 199 of this publication.

[6] See p. 19 of this publication.

TABLE 1. REPORT AND RECOMMENDATIONS OF THE UICC WORKING GROUP ON ASBESTOS AND CANCER: RECOMMENDATIONS ON PROBLEMS REQUIRING EPIDEMIOLOGICAL STUDY [a]

Recommendations on Problems Requiring Epidemiological Study

1. That the Importance of Fibre Type on the Risk of Developing Asbestosis, Carcinoma of the Lung, and Mesothelial and Other Tumours be Investigated.—International and intranational comparative studies of mining and other populations exposed to only one type of fibre are recommended. Among the countries in which and between which studies should, if possible, be made are Australia (crocidolite), Canada (chrysotile), Cyprus (chrysotile), Finland (anthophyllite), Italy (chrysotile), South Africa (amosite, chrysotile and crocidolite), the United States of America (chrysotile and tremolite), and the Union of Soviet Socialist Republics (chrysotile).

The studies of the effect of exposure to different types of fibre within a country are likely to be of special value, but studies of groups exposed to apparently similar fibres in different parts of the same country are also likely to be informative.

2. That the Relationship of Dust Dosage (including Concentration and Duration of Exposure), and the Composition and Physical State of the Dust to the Incidence of Asbestosis, Carcinoma of the Lung, Mesotheliomata and Other Cancers be Studied.—Comparative studies in factory populations in the asbestos textile and other manufacturing processes using asbestos are likely to be useful, especially when there are records of past dust measurements. In any prospective studies of new entrants, the measurement of dust by a standardised method should be regarded as an essential part of the investigation.

3. That the Effects of Removal from Further Exposure to Asbestos Dust be Investigated.—It is important to establish the subsequent morbidity and mortality from asbestosis, and the mortality from cancers associated with exposure to asbestos, in population groups no longer exposed to the dust.

4. That Further Investigations be made of Past and All Future Cases of Diffuse Mesothelial Tumours of the Pleura and Peritoneum to establish Any Association with Asbestos and Other Factors.—These tumours should be diagnosed on the criteria suggested by the Panel on Pathology (see below) and reviewed by a Panel of Pathologists with experience of these rare tumours. The tumour and lungs should be investigated for the presence of asbestos by physical and chemical methods (see below).

5. That Studies of Morbidity and Mortality be Extended to Asbestos-exposed Populations that have not so far been widely Investigated.

1. It is recommended that special attention be directed to surveys in (*a*) the insulating industry, including that in ships; (*b*) the asbestos cement industry; (*c*) asbestos products industry; and (*d*) other plants in which asbestos is regularly used, such as certain paper, paint and plastic factories.

2. It is also recommended that, since incidental exposure to asbestos dust may occur in certain trades and occupations, attention be directed to (*a*) handling and transporting asbestos; (*b*) the building industry; (*c*) pipe fitting; and (*d*) ship building and breaking.

3. It is recommended that surveys be made to study environmental and community exposures, including populations near mines and factories and elsewhere.

4. It is recommended that general population surveys be made nationally and internationally to establish by standardised methods, in areas of presumed high and low exposure to asbestos dust, the prevalence of asbestos bodies and fibres.

5. It is recommended that surveys of asbestosis in domestic and wild animals be extended to areas of high and low exposure.

6. Epidemiological Methods

1. *General*

(*a*) In addition to the usual information about the individual collected in such surveys, special attention should be directed to a detailed, social (including smoking habits), occupational, environmental and medical history from early childhood to elucidate any possible exposure to or association with any type of asbestos or other dusts. A study of the family unit or household may be of interest in view of the occasional reports of significant neighbourhood and household exposures.

(*b*) In view of the association between exposures to asbestos dust and pulmonary fibrosis and its complications, it is important to obtain as much information as possible about morbidity and mortality from *all* causes with particular attention to asbestosis, chronic bronchitis and emphysema, bronchiectasis, diffuse interstitial fibrosis, pneumonia, tuberculosis, cor pulmonale, carcinoma of the lung, diffuse mesothelial tumours of the pleura and peritoneum, gastro-intestinal tumours and ovarian tumours.

(*c*) The principal epidemiological surveys likely to be used are retrospective, cross-sectional and prospective, or a combination of these. In most of the surveys one or preferably more control groups will be needed. It is strongly urged that early and full consultation with statisticians be made at all stages from planning to an analysis of the findings.

2. *Clinical Criteria.*—The need to establish the minimal clinical information to be obtained in surveys of workers

[a] This table appeared first in the *British Journal of Industrial Medicine*, 22, 165–171, 1965 and is reproduced here by kind permission of the Editor, British Medical Association.

TABLE 1.—continued

exposed to asbestos was agreed. A small panel met informally after the main working party and their recommendations are as follows:

(*a*) Symptoms: It was agreed that in all surveys the presence or absence of cough, sputum, dyspnoea and chest pain should be recorded as a minimum. This should be recorded using a standardised questionnaire.

The British Medical Research Council Questionnaire on Respiratory Symptoms (1960), with the additional questions relating to chest pains in the WHO Questionnaire on Cardiovascular Disease (Rose, 1962), is suitable for this purpose; these questionnaires have been widely used internationally for interview surveys. The Cancer Society Questionnaire in the Cancer Prevention Study has been widely applied in the USA. It contains questions about a wide range of symptoms and diseases and was designed for self-completion.

(*b*) Signs: It was agreed that when physical examination was possible, the minimal observations should include the presence or absence of *clubbing of the fingers, cyanosis and basal rales in the chest.*

It was agreed that the measurement of sputum volume (first hour on rising) and degree of purulence recorded in a standard way (Miller, 1963) were useful in association with the questionnaires for assessing the prevalence of bronchitis, and this may be relevant to the disability caused by asbestosis.

The epidemiological usefulness of examinations of sputum for asbestos bodies and fibres is at present uncertain but needs investigation.

3. *Classification of Chest Radiographs of Asbestos-exposed Individuals.*—There is no international or national standardised classification of the radiological appearances of asbestosis. It is recommended that a scheme based if possible on an extension of the ILO Classification (1959) be developed.

Possible means of doing this were presented at the New York Academy of Sciences Symposium on the Biological Effects of Asbestos (1965) by Finnish, German, South African and British contributors. The aim should be to specify separately and record semi-quantitatively the principal radiological features seen in asbestos-exposed groups, but exposure to mixed types of dust is not uncommon, and the appearances may therefore include those caused in part by other pneumoconioses.

The classification should be purely descriptive of the radiological features and should not imply pathological change or the extent of disability. It is probable that the type and severity of alterations in radiological features, such as pleural plaques, etc., vary with the type of dust exposure and other factors so that a classification based on the principles in the ILO Classification for Pneumoconiosis, in which there is a semi-quantitative assessment of several qualitatively different types of abnormality, may be expected to be useful.

It is recommended that a working group be set up to develop and test a new international classification.

4. *Lung Function Assessment.*—The preferred lists of lung functions to be used will vary according to the type of survey and the facilities available. A list of minimal and additional tests likely to be of use is as follows:

Minimal
- Forced vital capacity (FVC)
- Forced expiratory volume over 1 sec. ($FEV_{1.0}$)

Additional
- Transfer factor (diffusing capacity) of lung for carbon monoxide—single breath method
- Lung compliance
- Standard exercise test
- Peak expiratory flow
- Airways resistance.

UICC Working Group led to a fruitful international effort to fill this gap. The efforts of the Group set up by the UICC, the Group of Radiologists working with the US Public Health Service, and the team under Professor J. C. McDonald at McGill produced and tested a scheme for the classification of all types of pneumoconiosis. This UICC/Cincinnati scheme was published in 1970 (UICC, 1970). Further discussions and tests of the classification between 1969 and 1971 led finally to the adoption of a scheme which achieved a satisfactory synthesis of the ILO 1968 and UICC/Cincinnati proposals to form a single ILO U/C 1971 scheme. This was recommended for international use by the ILO IVth Conference on Pneumoconiosis at Bucharest in September 1971. The standard films and the text of the scheme are now available from the ILO (ILO, 1972). Evidence of the usefulness of these proposals as a means of recording and quantifying the effects of different types of dust exposure in asbestos workers is given by McDonald [1], and Enterline & Weill [2]. The classification is now in use in prospective studies of asbestos workers in various countries.

Tests of lung function

Surveys of asbestos workers made since 1964 have usually employed a range of tests for measuring

[1] See p. 155 of this publication.
[2] See p. 179 of this publication.

changes in the different components of lung function, as was recommended in 1964. The results of such tests remain complementary to information obtained by the classification of the radiographs. Current knowledge about the discriminating powers of the different tests when applied to asbestos workers is reviewed by Becklake [1]. Information is accumulating to answer such questions as: What proportion of early or suspect cases of asbestosis will be missed if reliance is placed solely on radiographic screening of exposed groups? The usefulness and limitations of the methods for detecting the effects of asbestos in groups exposed to the dust is reviewed in this Symposium [2].

PROBLEMS

Fibre type

The importance of establishing whether the type of asbestos fibre inhaled is an important factor in the health risk was stressed in the 1964 recommendations, because of its implications for future prevention. Studies of mining populations exposed to only one type of fibre have now been made in Canada, Finland and Cyprus, and surveillance of the amosite and crocidolite mining areas has continued in South Africa (McDonald [3]; Sluis-Cremer & du Toit [4]; Ahlman *et al.*[5]; Meurman *et al.*[6]; Wagner *et al.*, 1971).

Extensive searches have continued in many countries to identify factory populations exposed to only one type of fibre. Some of these searches have been successful (Jones, 1973; Mancuso *et al.*, 1973; Selikoff, 1972), so that comparisons of apparently pure exposed groups are no longer limited to mining. Whether the differences in the health risks which have been demonstrated within the industry are thought to be more closely related to fibre type, dose of dust, or to the physical state of the dust, is one of the important topics discussed at the Conference.

Dust dosage

More information has usually been available concerning dust levels in factories than of those in mines, but this has often been scanty and not measured in a way which gave a good estimate of past exposure to the amount of respirable fibre. However, evidence for a dose-response relationship for asbestosis and for lung cancer and mesothelioma is now appearing as a result of recent surveys (McDonald [7]; Sluis-Cremer & du Toit [8]; Enterline & Weill [9]; McDonald [10]; Newhouse [11]). For the future, prospective studies are now under way in which it will be possible to relate dust levels measured in the appropriate manner to the attack rate of disease. However, there are probably more retrospective studies which could give useful information on this important problem.

The Conference provides an opportunity to review the information on dose-response relationships and to show whether or not there is now sufficient new biological evidence to propose alterations to current threshold limit values; also, whether these should differ with the type of fibre used or for different occupations in the industry.

EFFECT OF REMOVAL FROM FURTHER DUST EXPOSURE

Little progress has been achieved here. It is still not known whether there is functional or radiographic improvement after removal from further dust exposure at an early stage of asbestosis. It is generally agreed that progression occurs without further exposure when asbestosis has reached a degree of severity which permits confident diagnosis. Even so, the annual progression rate has not been established, nor which index of change is the most sensitive measure.

FACTORS ASSOCIATED WITH MESOTHELIOMAS

This has been a topic of intensive investigation and many of the papers in the Conference deal with this in one way or another. It is now clear that cigarette smoking is not an important factor. The type of fibre is important; however, none of the commercial types, with the possible exception of anthophyllite, is innocent.

[1] See p. 31 of this publication.
[2] See pp. 19–41 of this publication.
[3] See p. 155 of this publication.
[4] See p. 160 of this publication.
[5] See p. 165 of this publication.
[6] See p. 199 of this publication.
[7] See p. 155 of this publication.
[8] See p. 160 of this publication.
[9] See p. 179 of this publication.
[10] See p. 189 of this publication.
[11] See p. 203 of this publication.

The evidence before the Conference could lead to a useful statement summarising the relative importance of different types of fibre, and of other factors such as the subject's occupation in the industry. Some mesotheliomas are apparently unrelated to past exposure to asbestos; however, the proportion has varied quite widely in different surveys.

Techniques for identifying the type of fibre and its amount in the lung are developing rapidly. These have not yet been applied widely to large groups of cases of mesothelioma, but a start has been made (Pooley [1]). Papers by Oldham [2]; Pooley [3]; Hinson *et al.*[4]; and Davis & Gross [5] deal with the technical methods for doing this and give some of the results of international comparisons, using standardised methods. One of the gaps still to be filled is a clearer definition of the relative amounts and types of fibre encountered by occupationally exposed individuals, by those classed as environmentally exposed (Bohlig & Hain [6]), and by the general population. When this has been achieved, the margin of safety, or the lack of it, to the general population will be easier to establish.

The suggestion in the Recommendations that domestic and wild animals be studied as a means of comparing high and low areas of exposure does not seem to have been followed up.

PREVENTION

The incompleteness of retrospective epidemiological evidence on many points has not prevented the development of measures to improve working conditions in most parts of the industry in many countries. Threshold limit values have been lowered (for a discussion see Berry [7]), and detailed codes of practice have been issued for the many different uses of asbestos (Department of Employment and Productivity, 1969; Harries, 1971). National and occupational schemes of long-term periodic surveillance of employees are now in operation (Greenberg [8]). These will provide the earliest evidence of whether the improved working conditions ensure an acceptably low risk to health.

[1] See p. 222 of this publication.
[2] See p. 45 and p. 231 of this publication.
[3] See p. 50 of this publication.
[4] See p. 54 of this publication.
[5] See p. 238 of this publication.
[6] See p. 217 of this publication.
[7] See p. 145 of this publication.
[8] See p. 273 of this publication.

SUMMARY

Progress in epidemiology since 1964 is compared with the UICC Working Group recommendations on problems requiring further study. It is encouraging to find that most of the recommendations have been carried out in a wide range of studies, many of which are subjects of reports to this Conference.

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Progress in pathology/experimental pathology

J. C. WAGNER [1]

The recommendations relating to pathology and experimental pathology drawn up by the 1964 Working Group have been carried out by the Pathology sub-committee.

DIAGNOSIS OF ASBESTOSIS

The method for the assessment of the severity of asbestosis suggested in 1964 was tested by a number of workers and discussed by national and international panels. It was found to be unsatisfactory, and Dr K. F. W. Hinson, Professor H. Otto and Professor I. Webster were asked to draw up a new scheme. Several meetings took place, and Mr C. E. Rossiter, who had been concerned with the Radiological Classification, was asked to assist. A preliminary draft was presented to the European Panel, and the final scheme is discussed at this Conference (Hinson *et al.*[2]).

THE DETECTION AND SIGNIFICANCE OF ASBESTOS BODIES AND FIBRES

In the sputum

In 1964 it was recommended that the term "asbestos bodies" and not "asbestosis bodies" should be used. Doubt about the origin of these bodies has been raised, and some workers now feel that they should be referred to as "ferruginous bodies". This point should be clarified by this Conference. It is still considered that the quantitative assessment of bodies and fibres in sputum would not be a useful procedure: however, the investigations of Pooley [3] have made it possible to identify the types present.

In the lung tissue

A number of methods for the identification and quantitation of asbestos bodies and fibres in tissue have been investigated in a 12-centre investigation covering the United Kingdom, Kuopio in Finland, and Dresden (DDR). It was decided to use the thick, unstained section method developed by Meurman (1966); however, in a later study, a variety of histological preparations were compared with the maceration method developed by Gold (1968). An assessment of these methods is discussed by Oldham [4]. The possibility of using the maceration methods as a means of correlating the number of bodies and fibres found in the lung tissue with the severity of asbestosis and the possibly associated tumours has been considered. Major disadvantages of these methods are that fibre type cannot be distinguished and that a large number of the fibres present cannot be observed under the light microscope. Electron-microscopy is required to overcome both these difficulties and it is hoped that the feasibility of using combined methods will be discussed.

Pleural plaques

Further information about the pathology of pleural plaques was provided by Meurman (1966). The significance and composition of these lesions will be discussed by Jones & Sheers [5] and by Le Bouffant *et al.*[6].

THE DIAGNOSIS OF DIFFUSE MESOTHELIOMAS

(*a*) As recommended, an International Panel has been organised and national Panels have been

[1] MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, UK.

[2] See p. 54 of this publication.

[3] See p. 50 of this publication.

[4] See p. 231 of this publication.

[5] See p. 243 of this publication.

[6] See p. 249 of this publication.

formed in the UK, USA, South Africa and Canada; and the standardisation of diagnoses has been maintained by consultation among panels. The diagnostic criteria recommended in 1964 have been reassessed by McCaughey [1]. In addition to this, and in collaboration with Dr P. D. Oldham, he has produced a questionnaire to be used as an Observer Variation Trial on the histological diagnostic criteria. Using this questionnaire, the British Panel have studied a series of cases, and the results have been analysed by Dr Oldham. The questionnaire has been sent to other panels and is discussed at this Conference.

(*b*) *Electron microscopy*. The possibility of a specific diagnosis of mesotheliomas by electron-microscopy was first suggested by Echevarria (1967); this has been further studied by Suzuki *et al.*[2].

(*c*) *Histochemistry*. The use of histochemical methods in the diagnosis of mesotheliomas has been considered by all panels, but they have not been used by the British or South African Panels. However, some pathologists still find them of value, and Kannerstein and his colleagues have reviewed their value [3]. A considerable amount of work has been done on the chemical recognition and significance of hyaluronic acid in pleural fluid by Havez and his co-workers, and their findings are summarised in the same paper.

(*d*) *Exfoliative cytology*. This method of diagnosis has been studied by Dr Blanche Butler in Britain and the South African material has been reviewed by Dr Ann Berry. In their paper [4] they review the diagnostic criteria.

(*e*) It was recommended that use should be made of *tissue culture* in the diagnosis of the mesotheliomas. A start on this has been made by Dr K. T. Rajan and P. H. Evans who are using organ culture methods for the growth of adult human pleura and foetal lung[5].

Experimental collaborative studies have been carried out among British, American, South African and Russian workers. The standardisation of results has been made possible by using the reference samples of dusts prepared by the physicists (Timbrell[6]). The methods and some of the results of these investigations are presented by Wagner & Berry [7,8] and Stanton [9].

The members of the International Sub-Committee were: Dr J. Churg (USA), Prof D. Magner (Canada), Dr L. Meurman (Finland), Prof W. T. E. McCaughey (Eire), Prof B. Pernis (Italy), Prof I. Webster (South Africa), with Dr J. C. Wagner (UK) as Secretary. Later, Prof H. Otto (FDR), Dr H. T. Planteydt (Netherlands), Dr E. Roitzsch (DDR) and Prof L. Santi (Italy) agreed to join. In recent months, Dr M. Kannerstein has replaced Dr Churg as Secretary of the United States Committee.

[1] See p. 58 of this publication.
[2] See p. 74 of this publication.
[3] See p. 62 of this publication.
[4] See p. 68 of this publication.
[5] See p. 94 of this publication.
[6] See p. 13 of this publication.
[7] See p. 85 of this publication.
[8] See p. 285 of this publication.
[9] See p. 289 of this publication.

SUMMARY

The investigations undertaken by the Pathology sub-committee in implementing the recommendations of the 1964 Working Group are outlined. Most of these studies are presented in more detail at this Conference.

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Progress in physics and chemistry

V. TIMBRELL [1]

The UICC Working Group, 1964 recommended that standard reference samples of respirable particle size be prepared from commercially important types of asbestos, and that they should be characterised quantitatively by various methods. It also suggested methods of treating tissue sections and large samples of tissue, and recommended that methods for the identification and quantitative determination of asbestos fibres in the residues be further investigated.

REFERENCE SAMPLES OF ASBESTOS FOR EXPERIMENTAL WORK

Preparation and distribution

The standard reference samples were prepared in Johannesburg in 1966 (Timbrell & Rendall, 1972) by the collaborative effort of the Pneumoconiosis Unit (PU), Penarth and the Pneumoconiosis Research Unit (PRU), Johannesburg, with valuable assistance from Mr L. Kuyper, Mr D. French and Mr D. L. Schroenn and staff of the Cape Asbestos Insulations (Pty) Ltd, Benoni.

Dr J. C. Gilson obtained donations of 3000 lb quantities of commercial fibres of anthophyllite (Finland), amosite (Transvaal), crocidolite (North-Western Cape Province), chrysotile (Rhodesia) and chrysotile (Canada) from the following asbestos producers: Cape Asbestos Company Ltd; Pargas Kalkbergs Aktiebolag Finska Mineral; Turner and Newall Ltd; Johns-Manville Company Ltd and the other mines in the Quebec Asbestos Mining Association. The costs of preparation of the samples were borne by the Asbestosis Research Council of Great Britain, the Quebec Asbestos Mining Association, Canada and the Asbestosis Research Advisory Committee in South Africa. The Canadian sample was prepared from fibres supplied by eight mines, and the other samples from fibre from single mines. The grade of the starting commercial fibres was chosen on the advice of Dr R. Gaze of The Cape Asbestos Company, Ltd, London. Dr I. Webster arranged for the use of a mill newly developed by Mr R. F. Bourne of the Asbestos Grading Equipment (SA) (Pty) Ltd, Johannesburg. This choice of grade of fibre and mill proved extremely satisfactory.

Preliminary experiments to determine suitable methods of producing homogeneous samples of respirable particle size were carried out at PU, Penarth, where data had previously been obtained on the diameters (< 3 μm) and lengths (< 200 μm) of respirable asbestos fibres.

Dr R. J. Wells of PRU (SA) and Dr B. Commins of the Medical Research Council, Air Pollution Research Unit (UK) determined the oil content of samples from all bags of fibre to check that none contained more than the average for the consignment of that type of asbestos.

After separate blending of the contents of each of the 150 bags of raw material, 20% was allocated to pilot experiments, 40% to the preparation of the samples and 40% was put aside for possible future use. This means that large stocks (6000 lbs) of the original fibres are available in Johannesburg should they be required for the preparation of further samples.

To determine suitable settings for the mill, six pilot runs were made on each type of fibre, followed by determinations of particle size and of aerodynamic particle size in the products. The reference samples subsequently prepared (1000 lbs of each) contain approximately equal proportions (about 70%) of respirable particles, as measured by means of

[1] MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, UK.

Hexhlet-type instruments. The samples are thus much larger than those which the Working Group had in mind, but their extensive use in inhalation experiments has justified the preparation of such quantities.

The samples are distributed from PRU, Johannesburg, which holds the main stocks, and from PU, Penarth. These two centres have recorded 250 recipients. Additional recipients, whose number cannot be estimated precisely but which is known to be substantial, have obtained their supplies from colleagues. The request rate has risen steadily since 1966; there were 30 requests in the last six months. Even at this high rate of distribution, the samples, with careful use, should be available for several years.

Analysis and characterisation

As the result of a substantial international effort, the reference samples have been analysed and quantitatively characterised by all the methods listed by the Working Group, as well as by several other means. The examinations included: determination of composition by classical gravimetric analysis, atomic absorption spectrophotometry, neutron activation analysis and emission spectrophotometry; optical and electron microscopy for determination of fibre shape, fibre-length distribution, fibre-diameter distribution and optical properties; X-ray diffraction, electron diffraction and electron microprobe analyses; measurement of specific surface by nitrogen absorption and permeability methods; determination of oil content; investigations of homogeneity; studies of aerodynamic characteristics; investigations of the effect of heat treatment and reagents.

The Johannesburg distribution centre was able to provide data sheets on the main characteristics within months of the preparation of the samples, and it continues to circulate further information to users as it becomes available. Summaries of the data available in 1969 were presented at the Johannesburg conference (Timbrell, 1970; Rendall, 1970). A large amount of information on the characteristics of the samples and on their biological effects is now available in over 100 published articles. Chemical characteristics are discussed by Morgan & Cralley [1]; some physical features by Timbrell [2]; and biological properties by Wagner & Berry [3], Rajan & Evans [4], Wagner & Berry [5] and Stanton [6].

Waxes and oil

It was suggested that the waxes and oil that can be isolated from asbestos should be prepared and distributed. However, evidence from intrapleural inoculation experiments that oil-extracted asbestos has a similar carcinogenicity to that of the untreated fibre (Wagner & Berry, 1969) indicated that implementation of this proposal would provide no advantages.

Notification

Research workers were notified of the availability of the samples through publicity at meetings and in the scientific press, including a paper in the *International Journal of Cancer* (Timbrell *et al.*, 1968).

Applications

The standard samples and the idea behind them have been well received. The wide variety of applications found for them include their use:

(i) in studies on their biological activity in inoculation, tissue culture and organ culture experiments: also, as reference materials in similar investigations on glass, silicon carbide, aluminium oxide, nickel powder and other particulates;

(ii) in inhalation experiments to investigate their site of deposition, retention and biological activity: also, in physical studies to elucidate the influence of particle size and shape on the respirability of asbestos fibres;

(iii) in assessments of the applicability of various analytical techniques (such as X-ray diffraction and electron microprobe) for examination of asbestos-containing samples: the fact that the standard samples are of respirable particle size has been particularly pertinent to the evaluation of these techniques for analysis of the fine fibre recovered from tissue;

(iv) in the development of novel experimental techniques: for these purposes the fibres have been

(*a*) coated with polymer to study the effect on their biological activity;

(*b*) made radioactive to facilitate their detection in tissue sections;

[1] See p. 113 of this publication.
[2] See p. 295 of this publication.
[3] See p. 85 of this publication.
[4] See p. 94 of this publication.
[5] See p. 285 of this publication.
[6] See p. 289 of this publication.

(*c*) subjected to magnetic fields to demonstrate that different types of asbestos fibres exhibit different preferred orientations which can assist in their identification, sizing and quantitation in air or tissue samples;

(v) in testing respirators, dust sampling instruments and control equipment;

(vi) as demonstration materials and teaching aids by museums and similar establishments.

IDENTIFICATION AND QUANTITATIVE ASSESSMENT OF ASBESTOS IN TISSUES

Tissue sections

The Working Group suggested that tissue sections be ashed or treated with active oxygen and that the treated sections be examined by electron microscopy for recognition of sub-microscopic fibres. The present state of development of these techniques is reported by Pooley [1].

Large samples of tissue

It was suggested that methods of treating large samples of tissue with acetic acid, hydrogen peroxide and formamide be tried as they appeared to be superior to ashing. A similar technique, for extracting fibre from lung tissue but by digesting with KOH, has been described by Gold (1968). A comparison of this technique with that of the counting of asbestos bodies in histological sections is given by Oldham [2].

The Working Group recognised that the quantitative determination of asbestos in the residues obtained by digestion with reagents was difficult, but that it could be based on chemical analysis, X-ray diffraction or fibre counts. The problems in applying these examination techniques have proved to be severe, and no satisfactory method has been reported. The difficulties stem from scarcity of fibre in the residue, the substantial presence of minerals (silicates, phosphates) which grossly interfere with examination, and the fact that the fibres are often trapped in hard agglomerates. Even if the fibres can be freed, electron microscopy is still limited to relative quantitative assessment because of the many preparative steps involved in this type of examination. What is required is an improvement in the power of methods for discriminating between fibres and other materials. Centrifuging in liquids of suitable density may assist in concentrating the fibre and separating chrysotile from amphiboles, but these procedures are not devoid of problems.

In view of this absence of suitable quantitative methods it may be useful to mention an approach which has shown considerable potential in preliminary studies, but which, it must be stressed, requires confirmation of its general applicability to human tissue. The method is based on the fact that amphibole fibres in liquid suspensions exhibit preferred orientations in magnetic fields (Timbrell, 1972). Amosite and crocidolite, which resemble each other closely in most properties and are consequently difficult to distinguish, exhibit major differences in their magnetically-induced orientations; while anthophyllite resembles, but is not identical to, crocidolite in this respect. Chrysotile, and other particles in tissue residues, have not shown this effect. So, if a magnetic field is applied to a residue in liquid suspension, a laser beam will be strongly scattered in certain directions which depend on the type of particles present, at intensities which are related to the concentrations. Results of very recent physical studies with this technique indicate that it is extremely sensitive; in lungs of rats exposed in inhalation experiments to single types of amphibole (the residues from such lungs are virtually free from interfering substances), measurement of the scattered light should make it possible to assess 1 μg of fibre. Since these lungs may produce as much as 3 mg of residue, it should be possible to examine tiny pieces of tissue from the parenchyma and pleura and to determine quantitatively the spatial distribution of fibre. Examination of human tissues by this method is clearly more difficult, especially if they have been exposed to more than one type of asbestos. However, investigations of systematic procedures, which may include centrifuging as an initial step, have produced encouraging results on the quantitative determination of the different asbestos types that may be present. Studies are also in progress on the possibility of improving the discriminating power of X-ray diffraction and other examination techniques by applying magnetic fields to the residues.

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[1] See p. 50 of this publication.

[2] See p. 45 of this publication.

SUMMARY

The 1964 Working Group proposal for the preparation and characterisation of reference samples of asbestos has been carried out. This work is reviewed together with progress on the recommendation that methods for identification and quantitative determination of asbestos fibres in tissue be further investigated.

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ASSESSMENTS OF METHODS USED IN THE STUDIES OF THE BIOLOGICAL EFFECTS OF ASBESTOS. 1. CLINICAL

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Clinical signs

P. G. HARRIES [1]

INTRODUCTION

The clinical signs commonly ascribed to asbestosis are basal rales or crepitations (crackles), finger clubbing and cyanosis, but any of these signs may be absent even in advanced cases of the disease. All three signs have the disadvantage of considerable observer variation. Leathart (1960) reports disagreement among authors over the reported frequency of these signs in different series of patients; but Williams & Hugh-Jones (1960a) and Rossiter (1972) also describe the observer variation in the interpretation of chest radiographs in asbestos workers; and lung function tests are not without variability due to the operator, equipment and subject (Cotes, 1968; Bader *et al.*, 1965). It is essential, in assessing individual patients and also in epidemiological studies of persons exposed to asbestos, that all the available information be considered together, and that symptoms, signs, chest radiographs and lung function tests are not looked at in isolation from each other.

RALES, CREPITATIONS OR CRACKLES

Early descriptions of asbestosis report the presence of characteristic dry crackling sounds at the lung bases and in the axillae (Merewether, 1930; Wood & Gloyne, 1934). These non-musical, explosive sounds have been called "rales" or crepitations, and the term "rhonchi" has been applied to musical wheezing sounds. Forgacs (1967, 1969) recommends the adoption of the term "crackles" to describe non-musical explosive sounds (rales and crepitations), and "wheezes" for musical sounds (rhonchi).

Inspiratory crackles are associated with the re-expansion of areas of regional deflation in lungs stiffened by diffuse fibrosis from any cause, or with oedema. The crackles are usually heard at the base of the lung either throughout or only towards the end of inspiration. They sometimes occur in healthy patients during the first deep inspirations after recumbency, or when the lung is inflated after being partially deflated during periods of shallow breathing at rest. The subject should be asked to take a very deep breath and to cough, and if crackles persist after this manoeuvre the sign is positive.

The severity of lung involvement can be assessed by noting the extent of the area over which the sounds are heard, as they are usually heard at the lung bases or in the axillae early in the disease, spreading to most of the chest as the disease progresses. If the subject is examined lying first on one side and then on the other the crackles are often silenced in the uppermost parts of the lung and increased over the lower lung; Leathart (1968) suggests that they may only be detected in the early stages of asbestosis by using this procedure.

Pleural crackling is produced by friction between the two layers of pleura. The sounds are usually loud, unilateral, are heard over a small area of the chest wall and are often expiratory as well as inspiratory. The movement of the two layers of thickened pleura can sometimes be detected on palpation of the chest, and there is often impairment of the percussion note especially in the presence of a basal effusion.

It may sometimes be impossible to distinguish between pulmonary and pleural crackles, and they should be recorded simply as "crackles"; but for clinical purposes additional information obtained from the history of the illness and especially from the chest radiograph may help in attributing the sounds to the lung or pleura.

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RHONCHI OR WHEEZES

Musical sounds are usually associated with a degree of airways obstruction and should be recorded as wheezes. They are not usually associated with asbestosis, but when present they should be recorded. Forgacs (1967, 1971) gives an excellent account of the origin and classification of wheezes.

OBJECTIVE RECORDING OF LUNG SOUNDS

Although it is relatively easy to record lung sounds in visual form, there are difficulties in analysing the wave forms of wheezes. Tracings may be used to confirm the presence or absence of a repetitive pattern of crackles, but for most purposes the trained observer is probably better than the oscilloscope. Tape recordings of lung sounds have been used in a recent survey of men exposed to asbestos in shipyards (Murphy *et al.*, 1971).

FINGER CLUBBING

Finger clubbing is usually a late and inconstant feature of asbestosis. Clubbing includes an increased curvature of the nails both in the longitudinal and transverse axes, and an increased springiness of the nails on their beds, associated with a dusky red hyperaemia of the nail bed. There is often hypertrophy of the distal phalanx.

There is a great deal of observer variation associated with this sign. Regan *et al.* (1967) describe the measurement of the hyponychial angle from a lateral profile of a finger, usually the index finger of either hand; and Bentley & Cline (1970) have devised a method whereby a satisfactory profile from which to make the measurement can be easily obtained.

The hyponychial angle is that angle formed between two lines, one drawn from the distal skin crease to the cuticle and the other from the cuticle to the hyponychium (thickened stratum corneum of epidermis lying under the free edge of the nail). The measurement is performed on a lateral projection of the finger onto a plane surface.

Regan and her colleagues (1967) recorded a mean hyponychial angle of 187.0° in 18 normal subjects, and a mean value of 209.4° for 7 subjects with definite clinical finger clubbing. Mean values of 180.4° for normal controls and 184.6° for asbestos insulators were reported by Langlands *et al.* (1971); while Harries (1971a) found mean values of 183.4° for men with little asbestos exposure, and 187.5° in men heavily exposed to asbestos. Although these results suggested that there might be a relationship between hyponychial angle and exposure to asbestos, further analysis of Harries' data shows no significant relationship to exposure, but an increase with age and a decline with weight. The angle differed significantly among smoking groups (Oldham & Harries [1]).

There is clearly a need for further work to assess the usefulness of this measurement and to establish ranges of "normal values".

CYANOSIS

Cyanosis occurs in the later stages of asbestosis. The sign is positive when the mucous membrane inside the mouth, the tongue, and the skin show a bluish tinge. It is due to a reduction in blood oxygen saturation and a rise in the concentration of reduced haemoglobin. It is an unreliable sign, and cyanosis is often not recognised in subjects who have normal haemoglobin concentrations until the oxygen saturation has fallen to about 80%. In anaemia it may be absent despite marked hypoxaemia; it may be present in polycythaemia despite a normal oxygen saturation.

An objective quantitation of oxygen saturation can be made by measuring arterial oxygen tension, and this value has been shown to be reduced in asbestosis (Williams & Hugh-Jones, 1960b; Leathart, 1962; Thomson *et al.*, 1965; Bader *et al.*, 1965; Wallace & Langlands, 1971). A method of estimating arterial oxygen tension by puncture of the warmed ear lobe, which avoids the need for arterial puncture, has been described by Langlands & Wallace (1965).

MEASUREMENT OF CHEST EXPANSION

Reduction of chest expansion was described in the early papers on asbestosis (Wood & Gloyne, 1930; Merewether, 1930), and observation of the

[1] Unpublished data.

chest expansion during normal and maximal respiratory movements is of value in the clinical assessment of individual patients. However, the circumferential measurement of the chest in full inspiration and in full expiration involves so many errors, and the normal range of measurements is so wide, as to make this procedure of little value in epidemiological surveys of chronic chest disease (Moll & Wright, 1972).

ASBESTOS CORNS OR WARTS

Asbestos corns or warts occur frequently on the hands and forearms of asbestos insulators due to the penetration of the skin by sharp, brittle, asbestos fibres (Alden & Howell, 1944), or to the inoculation of the skin by sewing needles carrying minute asbestos fibres (Harries, 1971b). They are of no significance.

ASBESTOS BODIES OR FIBRES IN SPUTUM

The presence of asbestos bodies or fibres in sputum is of little clinical significance except that, like asbestos corns, they provide evidence of exposure to asbestos dust.

USE OF CLINICAL SIGNS IN STUDIES OF ASBESTOS DISEASE

The UICC (1965) Working Party recommended that all morbidity studies of asbestos diseases should record as a minimum the presence or absence of cough, sputum, dyspnoea, chest pain, basal rales (crackles), clubbing of the fingers and cyanosis, together with smoking habits and occupational history. The British Medical Research Council Questionnaire on Respiratory Symptoms (Medical Research Council, 1960), with additional questions relating to chest pains in the WHO Questionnaire on Cardiovascular Disease (Rose, 1962), were suggested as being suitable for standardised recording of the data; these recommendations have been followed by most of the epidemiological surveys carried out since 1965.

Attempts have been made to assess the relative importance of clinical signs and other variables in evaluating patients with established asbestosis (Williams & Hugh Jones, 1960b; Thomson *et al.*, 1965; Bader *et al.*, 1965), but most attention has been paid to the radiographic and lung function changes. A principal component analysis of sixteen variables—clinical signs, symptoms, radiographic changes and lung function tests—was used by Regan *et al.* (1971) to evaluate the relative power of those variables to separate health from lung disease, and asbestosis from chronic obstructive airways disease. Hyponychial angle (clubbing) ranked fifth and rales (crackles) seventh in relative power to separate asbestosis from chronic obstructive airways disease. A reasonable separation of those subjects with asbestosis from those with obstructive airways disease was shown when the component scores were plotted on vertical and horizontal axes, suggesting that use of the analysis might improve the precision of early diagnosis.

The effects of asbestos exposure in terms of clinical, radiological and lung function changes have also been studied by comparing the results in groups of asbestos workers with those in control groups, and this technique has also been used to study the prevalence of the disease among groups of workers with different degrees of dust exposure. Kleinfeld *et al.* (1966) found basal crackles in 19.6% and clubbing in 14% of asbestos workers, while the controls had none of these signs; however, they found poor correlation between lung function and clinical and radiological changes. Wallace & Langlands (1971) compared 50 insulators with 50 controls and found that higher proportions of insulators than controls had basal crackles, finger clubbing, breathlessness, cough and sputum. The authors suggest that those factors, together with radiographic abnormality and lung function tests showing a decrease in static lung volume, reduced arterial oxygen tension with an increased alveolar-arterial oxygen difference, are useful in the diagnosis of asbestosis.

A larger study of insulation workers (Langlands *et al.*, 1971) showed that 34% of men over 40 years old had basal crackles, compared with 6% of men under 40 years, but that there was little difference between these groups for the prevalence of wheezing. The hyponychial angle increased with age and was larger in men with radiographic lung field abnormality; and the angle for the left index finger was greater than that for the right. Basal crackles occurred more frequently in men with radiographic lung field abnormality, but there were no appreciable differences between men with only pleural abnormality and those with normal chest radiographs.

A comparison of 101 pipe coverers (insulators) with 94 "controls" in a shipbuilding yard with low asbestos dust concentrations showed basal crackles in 14.86% of insulators and 4.2% of controls (Murphy *et al.*, 1971). Clubbing (hyponychial angle > 198°) occurred in 19.8% of insulators and 5.3% of controls. Asbestosis was diagnosed when three or more of the following factors were present: dyspnoea on climbing stairs; basal crackles in two or more sites; clubbing; vital capacity < 80% predicted; and radiographic abnormality suggesting asbestosis. Using these criteria, 11 insulators were found to have "asbestosis"; and applying the same criteria to the data of Langlands *et al.* (1971), "asbestosis" occurred in 25 of the 252 men at Belfast.

A study of men exposed to asbestos in Devonport Dockyard also suggested relationships between the intensity and duration of exposure and the presence of lung crackles, clubbing and dyspnoea (Harries, 1971c). The clinical abnormalities were shown to be related to the degree of radiographic lung field abnormality; and men who also had pleural abnormality were found to have more symptoms and signs and lower lung function values than men without pleural abnormality (Harries, 1971d).

Careful observation of clinical signs, especially of basal lung crackles, may prove to be of value in the early diagnosis of progressive pulmonary fibrosis which often accompanies diffuse pleural fibrosis.

Further analyses of the Devonport data have shown that while basal lung crackles and dyspnoea (Grade 3) are associated with intensity and duration of exposure, wheeze and finger clubbing are not. The analysis suggests that of men with heavy continuous exposure to asbestos from age 15, 50% would have lung crackles by age 39; 50% of men with light continuous exposure from age 15 would have lung crackles by age 47; 50% of men with light intermittent exposure from age 15 would have lung crackles by age 65; and 50% of men with no exposure at all from age 15 would have lung crackles by about age 75 (Fig. 1).

Men with normal chest radiographs who had crackles, clubbing, dyspnoea or any combination of these abnormalities suggesting pulmonary fibrosis were found to have reduced static and dynamic lung volumes and gas transfer factor (single breath CO) and normal FEV/FVC ratios (Harries, 1971e). These observations add weight to the suggestion by Leathart (1968) that the presence of clinical signs, especially of lung crackles, may precede the detection of radiographic or lung function abnormalities in early asbestosis; and to that of Murphy *et al.* (1971) that minor degrees of clubbing, rales (crackles) and dyspnoea may reflect interstitial lung disease.

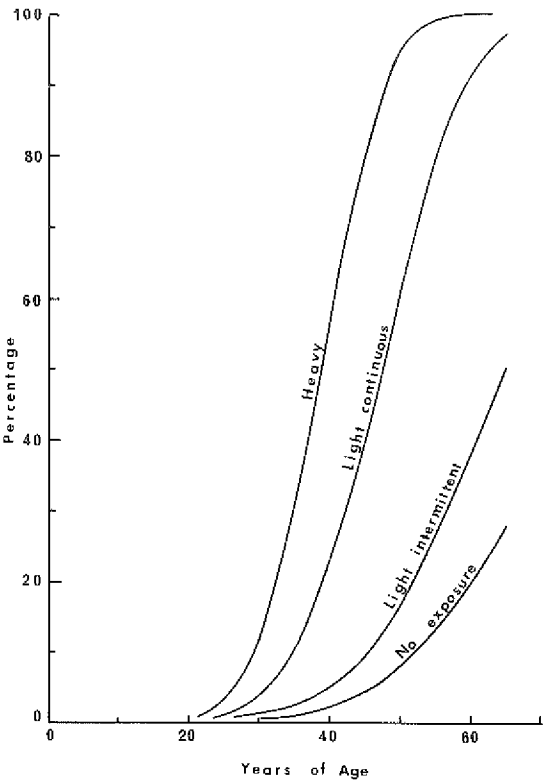

Fig. 1. Expected proportions of dockyard workers with basal lung crackles (rales) if consistently exposed to heavy; light continuous; light intermittent asbestos dust concentrations.

USE OF CLINICAL SIGNS IN DEFINING HYGIENE STANDARDS

Clinical signs can be used as measurable indices of response in persons exposed to asbestos dust, and from these indices a dose-response relationship may be estimated to provide the basis for recommending hygiene standards of acceptable levels of dust concentration.

Dreessen *et al.* (1938) suggested a threshold limit value of 5 million particles per cubic foot (mppcf)

after a survey of workers in the US asbestos textile industry had shown no cases of asbestosis in persons exposed to dust concentrations lower than this. Their recommendation was based mainly on radiographic evidence of the disease.

Basal lung crackles were considered to be the key sign by the sub-committee on asbestos hygiene standards of the British Occupational Hygiene Society (1968), since every subject with radiographic abnormality suggesting asbestosis had basal lung crackles, whereas some subjects with those sounds did not have radiographic abnormality. The prevalence of basal lung crackles in asbestos textile workers (16 of 290) was used to give a risk-exposure relationship, and the results were used to set a hygiene standard of that dose which gives a 1% risk of basal lung crackles for people with a working lifetime's exposure to the dust (Berry [1]).

DISCUSSION AND CONCLUSIONS

It would seem that of the clinical signs usually associated with asbestos disease, lung crackles are most closely associated with the intensity of exposure to asbestos, and with the severity of the disease as measured by lung function and radiographic changes. Finger clubbing appears, from some of the studies, to be related to the disease process, but there is evidence to suggest that smoking plays an important part in the development of this sign. Cyanosis is not an early feature of asbestos disease, and it is an unreliable sign; thus it is not of much use in epidemiological studies.

The UICC Working Party's recommendation (1965) for the recording of symptoms and signs should be followed in morbidity studies of asbestos diseases, the minimum requirement being the recording of the presence or absence of lung crackles and finger clubbing.

The prevalence of basal lung crackles in persons exposed to asbestos would appear to be of value in making estimates of exposure risks.

More work is required to establish the usefulness of the measurement of hyponychial angle, and to determine the relationship between this angle, finger clubbing, and the effects of age, smoking and asbestos exposure.

Further morbidity studies are required to examine in detail the natural history of all types of pleural abnormalities associated with asbestos exposure.

[1] See p. 145 of this publication.

SUMMARY

The clinical signs commonly associated with asbestosis, basal rales (crackles), finger clubbing and cyanosis are discussed, together with methods of objective measurement.

The uses made of the observation of these signs in recent studies of the biological effects of asbestos are reviewed, and it is suggested that basal rales (crackles) appears to be the most useful clinical sign in morbidity studies.

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Radiology

H. BOHLIG [1] & J. C. GILSON [2]

The chest radiograph remains the most important experimental tool for detecting and monitoring the biological effects of asbestos dust in the lungs.

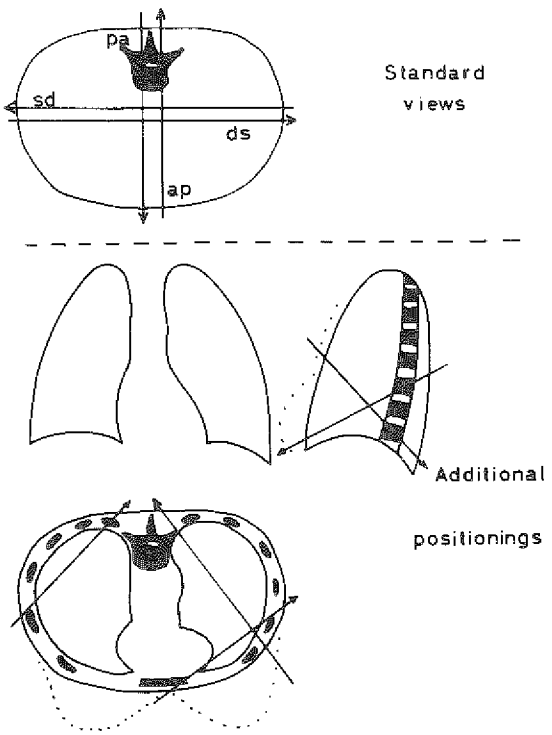

Fig. 1. Standard and additional views for radiological diagnosis of asbestosis.

It is used in two different ways: (1) for detailed study of the individual, and (2) for the biological monitoring of groups of subjects who risk exposure to asbestos dust (often in combination with other dusts). The techniques for these purposes differ. In the case of the individual, several views of the chest can be taken, and if necessary imperfect films can be repeated or additional views added (Fig. 1). For survey purposes one view (or, rarely, two) can be obtained, and only a very small recall rate is acceptable; also, the films have to be taken at a higher rate, so there is necessarily more automation in the processing.

The reporting on the films is also different. In the case of an individual the report is descriptive and interpretative, with little information regarding the separate features thought to be abnormal. In epidemiological studies it is more important to describe the extent and severity of each abnormality and to ensure comparability of classification with other studies.

Progress has been made recently in both uses of radiography. Firstly, the diversity of the radiographic appearances of asbestosis is more widely recognised, notably that bilateral pleural thickening and pleural calcification result more often than was previously recognised from asbestos inhalation (Bohlig *et al.*, 1960; Hurwitz, 1960; Fletcher & Edge, 1970). Secondly, there is now an internationally recommended classification of radiographic features for use in surveys (UICC, 1970).

TECHNIQUES OF CHEST RADIOGRAPHY

The aim is to produce a picture "in which the lung is shown in the greatest detail" (Jacobson, 1970), but there is no general agreement on a single technique for doing this. A number of quite different techniques will produce excellent films if sufficient attention to detail is given at all stages and if the right balance of exposure and development is

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employed. Two ranges of kilovoltage are in use; one extends from about 60 to 80 kV, the other—the high kV technique—from 110 to 140 kV.

In the high kV method, it is necessary to use lead grids to reduce scatter; these grids are also useful at lower kV for improving the detection of pneumoconiosis in subjects who are thick or fat (National Coal Board, 1971). It is now possible to obtain grids which are so fine that they make an indiscernible shadow on the film and thus do not interfere with the detection of small opacities, even those of only 1 mm or so in size. Such grids are, however, expensive and easily damaged. The high kV technique permits better views of the periphery of the lung, and the rib shadows are less prominent; however, small areas of calcification may be less easily detected, because in the lung absorption of X-rays by calcium falls off more steeply with rise of kV than it does in other tissues (Tuddenham *et al.*, 1954). The high kV technique is in use in hospitals in some countries, and it is claimed that the average standard of chest film is raised (Jacobson *et al.*, 1970). However, it is the standard kV technique which has been used almost universally for films so far classified in epidemiological studies, and few mobile X-ray sets are equipped to take high kV films. Table 1 summarises the differences between the two techniques.

Table 1. Good (++) and poor (−) possibilities of interpreting radiologic features with the high-kV and the low-kV techniques

Radiological visualisation of asbestos dust inhalation sequelae	High-kV technique	Low-kV technique
Asbestosis of the lung	++	−
Pleural features:		
thickening	++	−
hyaline plaques	++	−
calcified plaques	−	++
Lung cancer	+	+
Mesothelioma	+	+
Other complications such as pneumonia, TB, etc.	−	++

It is necessary now to test the two methods under field conditions, both to compare the quality of the films obtained and to discover if the level of kV itself effects changes in the features used in classification of the films. The newer methods of rapid processing using higher temperatures and rollers may produce more consistent films, but these may not always be of as high a quality as were obtained with the earlier automatic processors which did not compress the emulsion. The standard speed films may also produce better pictures than do high speed films, provided the length of exposure can be kept short (less than about 0.08 seconds).

SIZE OF FILM

Recent investigation of the size and shape of films required for routine surveys of pneumoconiosis in the United Kingdom (Audsley *et al.*, 1970) has shown that the commonly used sizes of films, 14 × 14 inch (355 × 355 mm) and 14 × 17 inch (355 × 432 mm), are far from optimal. The 14-inch film is too narrow and too short. Thirty-four per cent of subjects were not fully covered for width, and seventeen per cent not covered for height. The film size should be increased to 400 × 400 mm, in which case less than two per cent of subjects would not be fully covered by a single film. There is a particular indication for using these larger films in asbestos-exposed workers since the earliest changes may be at the bases of the lungs and on the lateral chest walls.

In general, miniature films (100 mm) and photofluoroscopy have not been found satisfactory for detecting the presence of the lower categories of rounded small opacities. Whether these methods are adequate for the detection of irregular small opacities and pleural thickening is not at present clear; trials are now in progress to compare miniature and full-sized films of asbestos-exposed populations. At present, therefore, full-sized films are recommended.

What is the appearance of an ideal film for assessing pneumoconiosis? Questions on the acceptability of films were included in a study of intra- and inter-observer variation during the development and testing of the UICC classification (Rossiter, 1972). The results showed that the agreement among the observers about whether the film was acceptable or not was less good than was the agreement for the recording of features in the classification. There is at present a wide variation of opinion among experts as to what is an ideal film. To establish a concurrence more tests are needed, for example, recording preferences in paired comparisons of films of different technique. To decide on an optimum technique, the range of preferred techniques could then be tested by studies of inter- and intra-observer

variation in classifying sets of films. Without such studies it will not be possible to define more exactly "the film in which the lung is shown in greatest detail"; to this might be added, ". . . in all six zones and along the lateral chest walls", since in asbestos-exposed workers it is in the bases and along the chest wall that the earliest changes tend to occur.

The importance of consistency of technique is great in any survey, but it is even more so when monitoring populations over a period of time, as is necessary to detect early changes due to dust. Radiologists could perhaps learn from physiologists to make more precise measurements of the accuracy of their methods.

INTERPRETATION AND CLASSIFICATION

When reporting an individual's film it is usual to comment on the abnormality present and to interpret any changes in pathological terms—fibrosis, possible cancers, etc.—and also to mention differential diagnoses. For epidemiological purposes such reports contain too little information about the type and extent of the abnormal features and too much interpretation. One of the reasons for using films in epidemiological studies is to discover more precisely what shadows signify in terms of morbidity and mortality, and how they relate to measures of exposure and tests of lung function.

In practice, films taken during epidemiological surveys usually have to be read in both ways: a clinical reading, which helps decide what action is required concerning the individual; and one or more readings by several observers to relate the information collected in the survey into a systematic classification.

The need for international agreement on the classification of the radiographic appearances of pneumoconioses has long been recognised (Gilson, 1972). Proposals of this kind concerning silicosis were made at the first ILO Conference on Pneumoconiosis in 1930. At the third ILO Conference in 1950 the proposals were extended to cover coal workers' pneumoconiosis as well, and new principles of classification were evolved. The classification was now primarily descriptive and no longer interpretative in terms of pathology or disability of the individual. The rounded opacities were separated into small and large, and their size and profusion were specified. Tests of inter- and intra-observer variation regarding the classifications were made for the first time. This ILO 1950 classification was further developed to widen its cover and increase the precision of its definitions by the US Public Health Service group of radiologists (Ashford

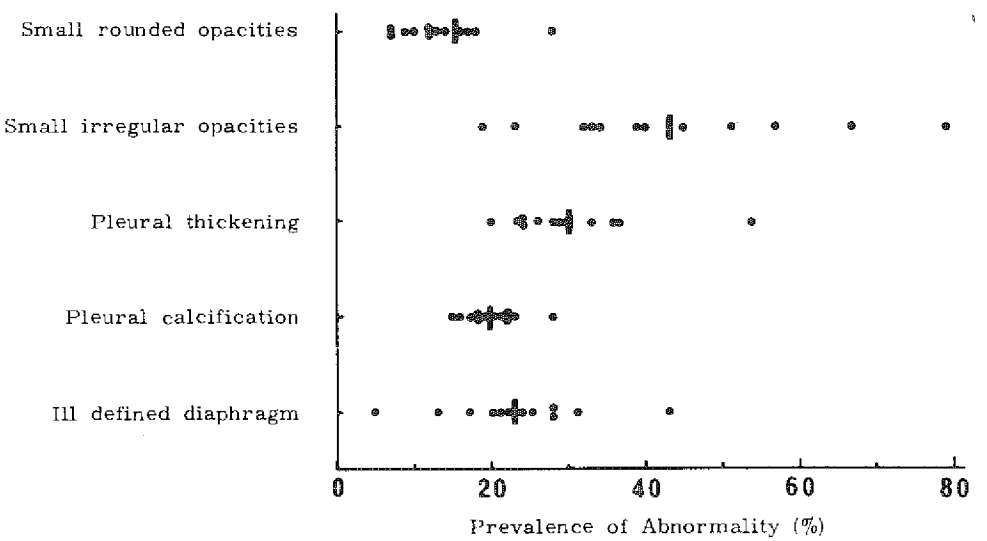

Fig. 2. Prevalence of abnormality for 5 indices of radiological change. (Mean and range for 100 films read by 12 readers.)

& Enterline, 1966), and by the ILO Expert Committee, 1958 (ILO, 1959). However, the classification still did not cover the changes seen following exposure to asbestos.

Epidemiological studies of asbestosis were stimulated by the first International Conference on the Biological Effects of Asbestos (NYAS, 1965) and by the UICC (1965) Working Group on Asbestos and Cancer. These led to a series of national and international meetings and to the evolution of the UICC/Cincinnati classification of the radiographic appearances of pneumoconioses. This classification was based on the ILO 1958 proposals and used its definitions and standard films for the large and small rounded opacities (with some modifications), but new categories of "irregular small opacities" and of pleural thickening and pleural calcification were added. Tests of inter- and intra-observer variation were made internationally. These showed that some of the features of the classification had a much smaller variation than others (Fig. 2), but that the precision of the scheme as a whole was comparable with that reported for earlier classifications and for those in other fields of clinical assessment (Rossiter, 1972). Tests using the earlier ILO classifications have shown that inter- and intra-observer variation is reduced with experience and within a group of readers classifying survey films. Thus, in practice one could expect better agreement than that seen in Figure 2.

Between 1967 and 1971 the classification was used for a number of epidemiological studies. As a result, in 1971, some further improvements were proposed to remove ambiguities in the definitions. Finally, at the fourth ILO Pneumoconiosis Conference in Bucharest in 1971 it was agreed to fuse the UICC/Cincinnati classification with the ILO 1968 proposals to produce a single international scheme—the ILO U/C classification 1971. The outline of this scheme is shown in Table 2. Full details of the text and the standard films are now being prepared and will be issued by the ILO (1972) and published in English, French and German, with illustrations, elsewhere (Jacobson & Lainhart, 1972).

The epidemiologist is interested in placing the film as accurately as possible in a continuum which extends from "complete normality" at one end to the most severe degree of abnormality at the other. It is also desirable to do this for each feature which can be separated with reasonable assurance—for example, pleural thickening, rounded or irregular small opacities, large opacities, etc. Thus the continuum is arbitrarily divided into formal categories, e.g., 0 (normal), and categories 1, 2 and 3 for small opacities. However, it has been shown

Table 2. Outline of classification

Feature	Short classification	Extended classification
No pneumoconiosis	0	Rounded 0/-, 0/0, 0/1 Irregular
PNEUMOCONIOSIS		
SMALL OPACITIES		
Rounded		
Profusion [a]	1, 2, 3	1/0, 1/1, 1/2; 2/1, 2/2, 2/3; 3/2, 3/3, 3/4
Type	p, q(m), r(n)	p, q(m), r(n)
Extent	—	zones: Right, Left; Upper, Middle, Lower
Irregular		
Profusion [a]	1, 2, 3	1/0, 1/1, 1/2; 2/1, 2/2, 2/3; 3/2, 3/3, 3/4
Type	s, t, u	s, t, u
Extent	—	zones: Right, Left; Upper, Middle, Lower
LARGE OPACITIES		
Size	A, B, C	A, B, C
Type	—	wd (well defined), id (ill defined)
PLEURAL THICKENING		
Costophrenic angle	—	Right, Left
Walls and diaphragm		
Site		Right, Left
Width	pl	a, b, c
Extent		1, 2
DIAPHRAGM OUTLINE	—	Ill defined: Right, Left
CARDIAC OUTLINE	—	Ill defined: 1, 2, 3
PLEURAL CALCIFICATION		
Site	plc	Walls, Diaphragm, Other; Right, Left
Extent		Length: 1, 2, 3

SYMBOLS

ax = coalescence of small rounded pneumoconiotic opacities
bu = bullae
ca = cancer of lung or pleura
cn = calcification of small pneumoconiotic opacities
co = abnormality of cardiac size or shape
cp = cor pulmonale
cv = cavity
di = marked distortion of the intra-thoracic organs
ef = effusion
em = marked emphysema
es = eggshell calcification of hilar or mediastinal lymph nodes
hi = enlargement of hilar or mediastinal lymph nodes
ho = honeycomb lung
k = septal (Kerley) lines
od = other significant disease. This includes disease not related to dust exposure, e.g., surgical or traumatic damage to chest walls, bronchiectasis, etc.
pq = pleural plaque (uncalcified)
px = pneumothorax
rl = rheumatoid pneumoconiosis (Caplan's syndrome)
tba = tuberculosis, probably active
tbu = tuberculosis, activity uncertain

[a] Use of 12-point scale for small opacities.

(Liddell, 1963) that for the rounded small opacities observers can do better than this, for it is possible to place the film either in the middle or towards the upper or lower ends of each category and thus achieve more information. This leads to the use of a 12-point scale (Table 2). The same procedure is recommended for the irregular small opacities, but more tests are required to show how much extra information is actually derived by using the 12-point scale in this part of the classification.

In a number of countries training courses have been held, or are planned, to make the classification familiar to a wide range of physicians, and thus to provide groups consistently able to use it for surveillance and for other purposes (Jacobson & Gilson, 1972).

The first results of using the classification are appearing in studies of lung function in relation to the duration and intensity of past exposure to asbestos (Becklake *et al.*, 1972; Rossiter *et al.*, 1972). These show the usefulness of separating rounded and irregular opacities, and pleural thickening and calcification. Rounded opacities are more prevalent where the subjects have been exposed not only to asbestos but also to other mineral dusts, and in particular silica. The rounded small opacities signify more clearly than do irregular small opacities the extent of past dust exposure; in coal workers rounded opacities relate well to the quantity and composition of the dust within the lung. Irregular opacities have not yet been related to the amount of asbestos or of other dusts in the lung, and they may well be an index of tissue response rather than of dust. For this reason it seems important to classify separately the rounded and irregular opacities until more is known about their correlates. Several studies are now under way to investigate the significance of the pleural changes alone or in combination with parenchymal changes in terms of prognosis, altered lung function, and the incidence of mesothelial tumours (Woitowitz *et al.*, 1970; Bohlig, 1972; Wagner *et al.*, 1971). Possible ways of numerically scoring the results obtained by classifying radiographs have been discussed by Oldham (1971) and by Jacobsen *et al.* (1971) for application to epidemiological studies.

SUMMARY

Radiology remains the most important experimental tool for detecting and monitoring the effects of asbestos on the lung. It is now generally recognised that past exposure to asbestos results more frequently than was previously thought in bilateral pleural thickening and calcification, and that these may be the only signs. Good quality radiographs can be obtained by several techniques. High kilovoltage (110–140 kV) may be preferable; it is claimed to produce better films in hospital practice, but so far it has been seldom used for epidemiological studies. Comparative tests of different techniques under field conditions are needed. It is now possible to classify the radiographs descriptively so that the separate features can be recorded semiquantitatively, using the ILO U/C international classification 1971. Results so far obtained using this classification indicate that it is a practical scheme and a useful epidemiological tool.

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Lung function

MARGARET R. BECKLAKE [1]

Assessment of the effects of asbestos exposure on lung function may be required for various purposes, of which the following are the most common:

(*a*) *Epidemiological studies*, usually aimed at detecting differences between asbestos-exposed and non-exposed populations in terms of lung function measurements with a view to defining whether or not a health hazard exists. Some of these studies have attempted to establish a dose relationship; others were undertaken to try to define "the earliest detectable effects" of asbestos dust exposure, so as to be able to suggest health surveillance measures for the exposed workers.

(*b*) *Studies examining the relationship of impaired lung function tests and "organ failure"*; evaluation along these lines also has practical importance for compensating the exposed worker.

(*c*) *Descriptive studies of lung function in established asbestosis*, usually aimed at answering the question: what patho-physiological changes characterise this condition?

Choice of the methods of measurement will depend on the purpose of the study, and it is therefore not surprising that almost all of the many methods developed over the past 25 years for the study of overall and regional lung function have been used at one time or another to assess the biological effects of asbestos exposure on lung function. An assessment of methods, such as this symposium calls for, can therefore only be made in the light of these different objectives.

EPIDEMIOLOGICAL STUDIES

A number of studies, in which comparison is made between an asbestos-exposed population and a suitable, non-exposed group (Table 1), have had as their objective an evaluation of the risk to health in a particular industry. It is evident that the results of such studies will depend "crucially on the selection of subjects" (Worth *et al.*, 1970) as well as on the health risk in the particular plant or industry concerned. It is therefore of interest that the prevalence of changes in the chest radiograph in exposed individuals is not very different in the four studies listed (28.5 to 43.6%). Furthermore, all four studies have led to somewhat similar conclusions about which measurements of lung function are most likely to distinguish the exposed individuals from those not so exposed, namely a lung volume measurement (either vital capacity (VC) or residual volume (RV)) and a measurement of gas exchange; any future studies planned to evaluate the health risk to a particular group of workers should, therefore, include these measurements.

Other studies, also epidemiologic in nature, have had as their objective an evaluation of health risk in relation to dust dosage (Table 2). Dust dosage has been measured on the basis of number of years of service in an industry (Bader *et al.*, 1970); by a rating of the job of the exposed individual (Harries, 1971); and by a risk index calculated from dust levels and duration of exposure (Becklake *et al.*, 1972; Woitowitz, 1972). Again, the conclusions are surprisingly similar, considering the differences in the industries studied and in the methods of evaluation of exposure. Thus, three of these studies (Becklake *et al.*, 1972; Harries, 1971; Woitowitz, 1972) led the authors to conclude that a dust–dose relationship is demonstrable in respect of VC or IC (inspiratory capacity), but not in respect of tests of gas exchange. In the fourth study (Bader *et al.*, 1970) a dust–dose relationship was found in respect of reduced lung function, which was considered to be present when VC was less than 75% predicted,

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Table 1. Epidemiologic studies comparing asbestos-exposed with non-exposed or slightly exposed populations

Reference	Populations studied	Exposure	*n*	% with chest X-ray 1/0 or more	Lung function tests				% with dyspnoea
					VC (litres or % expected)	RV	(FEV_1/FVC)%	Gas exchange A–a O_2 D_L difference	
Kleinfeld *et al.*, 1966	Asbestos insulators	18–40 yrs	56	28.5	81.1%*	90.1%	77.0	24.0*	16.0
	Non-exposed group	—	50	—	101.1%	96.7%	78.7	31.6	10.0
Murphy *et al.*, 1971 [b]	Pipe coverers	8–10.0 mppcf [a]	101	43.6	3.9*		76.9		37.6
	Shipyard workers	—	94	22.2	4.3		83.7		24.3
Ferris *et al.*, 1971	Pipe coverers (ship repair)	0.6–132 mppcf [a]	61	35.5	3.75		78.5	21.8	increased prevalence in pipe coverers, when standardised for age and smoking
		0.01–25 fibres/ml	63	8.0	3.75		76.5	23.2	
	Pipe fitters	? slight	61	9.8	3.95		76.5	23.6	
	Welders	? slight							
Wallace & Langlands, 1971 [b]	Insulators	4–48 yrs	50	40.0	4.17	1.55*	72.4	20.0 24.0*	16.0
	City employees	—	41	2.0	4.36	1.78	73.6	21.1 15.5	15.0

[a] Millions of particles per cubic foot.
[b] X-ray changes include those listed as "slight" or "early", as well as those considered definite.
* Tests considered by the authors to distinguish the exposed workers.

and FEV_1 (forced expiratory volume, 1 second) less than 70% of VC.

These studies support the view that VC (or FVC (forced vital capacity)) would be an appropriate measurement for the annual health surveillance of asbestos-exposed workers. A similar conclusion has been reached on empirical grounds by others who have had the opportunity to make long-term studies of exposed workers (Bader *et al.*, 1964; Gandevia, 1967).

THE "EARLY" EFFECTS OF ASBESTOS EXPOSURE ON LUNG FUNCTION

The biological effects of asbestos exposure have customarily been studied using tests of lung function and the chest radiograph (Tables 1 & 2), and reports frequently comment on the interrelationship of these two methods of measurement. While such interrelationships do not throw direct light on, for instance, the prevalence of effects on health and health hazards, they have led to some interesting speculation in regard to their relative sensitivity in detecting the early effects of exposure.

Thus, Williams & Hugh-Jones (1960) concluded that changes in pulmonary function, particularly in the diffusing capacity, may precede clinical or radiological signs. Along the same lines Bader *et al.* (1970) concluded from an analysis of their case material that although there was a correlation between marked radiographic and functional changes, such correlation did not exist in the earlier years of exposure, when function changes (VC less than 70% predicted, and/or FEV_1 less than 70% VC) occurred in 32% and radiographic changes (Grade 2–3) only in less than 5%. This conclusion is based on the assumption that levels of "significance" for the radiologic and the lung function changes can be equated: obviously, if Grade 1 radiologic changes had been considered, the conclusions might have been different.

The suggestion was made by Leathart (1968) and by Williams & Hugh-Jones (1960) that diffusing capacity might be particularly sensitive to the early effects of asbestos dust exposure, but this has not been confirmed in subsequent studies (Pilat *et al.*, 1971), including those of an epidemiological nature, on larger samples of working populations. These showed (see Table 3 and discussion above) other

Table 2. Epidemiologic studies exploring a dust–dose relationship

Reference	Population studied	Dust groups	*n*	% with chest X-ray changes	Lung function tests					
					Lung vols.		Flow rates		Gas exchange	
Bader *et al.*, 1970	Male members of an asbestos workers' union *n* = 598	**yrs of service**		**Grade 2–3**	**% with reduced function** [a]					
		0–	26	—	—					
		5–	60	3	27					
		10–	271	4	31					
		20–	83	22	43					
		30–	112	45	48					
		40+	46	53	46					
Harries, 1971	Naval dockyard workers *n* = 420	**occupation**		**1 or more**	**VC***	**RV**	**(FEV_1/VC)%**		**T_L**	
		intermittent	198	3	4.04	1.83	75.9		27.5	
		storemen, etc.	44	13	3.93	1.83	76.2		27.2	
		laggers	123	8	3.93	1.55	77.6		25.6	
		sprayers	55	24	3.43	1.96	71.7		20.9	
Woitowitz, 1972	Asbestos factory workers with exposure index > 1 *n* = 351	**exposure index** [b]			**correlation (r) with exposure index G × t** [b]					
					VC		**Rt (cm/l/sec)** [c]		**PaO_2 (exercise)** [d]	
		♂ < 40 yrs	106		−0.28*		−0.04		no relationship to G × t	
		40 yrs+	131		−0.24*		−0.25*			
		♀ < 40 yrs	48		−0.24*		−0.03			
		40 yrs+	66		−0.08		−0.01			
Becklake *et al.*, 1972	Chrysotile mine and mill workers *n*1 = 149 (non-smokers) *n*2 = 866 (smokers)	**dust index** [e]		1/0+	Pleural changes	Both	**IC*** [f]		**(FEV_1/FVC)%**		**D_{LCO} (SB)** [f]	
		< 10	91	3.3	1.1	0	3.12	2.31	79.9	78.8	31.1	28.8
		10–	455	3.2	13.4	2.6	2.79	2.75	80.6	78.2	25.1	28.7
		100–	159	6.2	20.5	3.7	2.83	2.65	81.9	77.8	28.3	28.7
		200–	134	9.8	22.0	8.3	2.63	2.61	82.5	78.1	25.1	30.1
		400–800	176	c. 6.1	20.2	14.2	2.51	2.48	81.6	75.3	28.4	27.7

* Considered significant, or to show a dose relationship.
[a] VC < 75% predicted and (FEV_1/VC)% < 70%.
[b] G × t, where G = dust rating of job and t = time (yrs) in that job.
[c] Rt = respiratory resistance (body plethysmograph).
[d] Arterialised capillary blood.
[e] Cumulative value of dust level × time (Gibbs & Lachance, 1972).
[f] Standardised for age, height, weight, non-smokers and smokers tested separately.

measurements (usually VC or IC) to be more sensitive to the lower dust doses. Indeed, an analysis of our own data in relation to radiologic changes (Becklake *et al.*, 1970) indicates that changes in $D_{L_{CO}}$ (SB) (lung diffusing capacity of CO, single breath), as well as in $D_{L_{CO}}$ (SS) (steady state) at rest and with exercise, occur only when radiologic changes indicate diffuse interstitial disease of moderate degree, i.e., 1/2 or more on the UICC classification (UICC, 1970).

In an attempt to distinguish the early effects of asbestos dust exposure, we also studied 25 workers with different degrees of exposure, all of whom had normal chest radiographs (UICC classification 0/0). Those with heavier dust exposure were found to have an increase in upstream resistance and in static compliance, changes which are compatible with dust deposition in and around small airways (Jodoin *et al.*, 1971). If these findings are substantiated by others, it is possible that more generally applicable techniques reflecting the state of the small airways, such as measurements of "closing volume" (Anthonisen *et al.*, 1969; Dollfuss *et al.*, 1967; Leblanc *et al.*, 1970), or analysis of the maximal expiratory flow volume curve such as has been proposed by Bouhuys (1970) will be of value in the detection of the early effects of asbestos dust exposure.

STUDIES EXAMINING THE RELATIONSHIP OF IMPAIRED LUNG FUNCTION TESTS AND "ORGAN FAILURE"

The biological effects of asbestos exposure on the lung are of social importance in so far as they affect the overall health and function of the exposed individual. Thus it is important to establish the interrelationship between organ malfunction, or failure, on the one hand, and overall health and function of the individual on the other.

In terms of the lung, *organ malfunction* is reflected by impairment in what might be called the descriptive measurements of size (lung volumes) and in other measurements reflecting or related to pulmonary mechanical properties. *Organ failure* is generally considered to be present only when the gas exchange function is impaired (Bates *et al.*, 1971). The great reserves of pulmonary function allow for a normal range of performance from a resting O_2 consumption of, for example, 0.25 l/min up to what may be a 10- to 15-fold increase with hard exertion. The ability to maintain normal levels of arterial blood gases, even with exercise, does not, however, necessarily exclude the presence of organ failure, since this may only be achieved by increasing minute ventilation (detectable from increases in the dead-space ventilation, or $V_D/V_T\%$) or by increasing the driving pressure (increased alveolo–arterial O_2 tension differences). Measurement of CO transfer (or diffusing capacity) is frequently substituted for measurement of O_2 exchange, for reasons of technical simplicity. Thus, there are many ways in which organ failure of the lung can be tested, from a complex and detailed system for examining regional ventilation-perfusion relationships (Read & Williams, 1959) and the measurement of A–a O_2 differences (Wright, 1955; Wallace & Langlands, 1971) to the measurement of the CO diffusion during a single breath-hold (see Tables 1 and 2).

Many of these techniques have been applied at one time or another to the study of the biological effects of asbestos (see Tables 1 and 2), and several authors comment on the discrepancy between the chief symptom of "organ failure" (breathlessness) and objective measurements indicating organ malfunction and/or organ failure, particularly in the individual case (Bader *et al.*, 1970; Williams & Hugh-Jones, 1960; Wright, 1955). This discrepancy is also evident in some epidemiologic studies (Kleinfeld *et al.*, 1966; Wallace & Langlands, 1971) which showed significant differences between exposed and non-exposed groups in terms of organ dysfunction (VC and D_L), but not in the prevalence of dyspnoea; in other studies this discrepancy was not evident (Ferris *et al.*, 1971; Murphy *et al.*, 1971). Likewise, in our own data on the chrysotile mine and mill workers of Quebec (Becklake *et al.*, 1972; McDonald *et al.*, 1972) dust dose related to breathlessness on exercise and to lung malfunction (specifically IC), but not to the overall profile of exercise adaptation. Woitowitz (1972) also failed to relate dust dose to the arterial O_2 tension profile at work loads up to 90 watts; and Harries (1971) related dust dose to VC but not to T_L (the transfer factor, or D_{CO}-single breath). Commenting on his findings, Woitowitz (1972) concluded that the lung reserve was adequate to maintain its gas-exchange function in the face of considerable malfunction as reflected by other tests, a conclusion in keeping with the experience of others (Bates *et al.*, 1971; Becklake, 1965, 1971).

Table 3. Function profiles in 305 reported cases of asbestosis

Reference	*n*		Lung function profile [a]					
	Total	No. classif.	Restrictive	Alveolo-capillary block	Obstructive	Mixed	Normal	Other disease present
Bastenier *et al.*, 1955	9	9	2		2	5		
Gernez-Rieux *et al.*, 1954	3	3	2		1			
Gaffuri & Berra, 1957	30	29	8		8	12	1	
Marks *et al.*, 1957	1	1				1		
Read & Williams, 1959	22	22	13		9			
Williams & Hugh-Jones, 1960	21	18	4	1	7	1	2	3
Heard & Williams, 1961	6	6	2			3		1
Bader *et al.*, 1961	17	17	6	6	1	4		
Thomson *et al.*, 1961	39	39	13	6	4	12		4
Boilinelli *et al.*, 1963	1	1	1					
De Rosa *et al.*, 1964	85	42	28		5	3	6	
Bjure *et al.*, 1964	8	8	5		2	1		
Sartorelli *et al.*, 1965	18	16	5		11			
Vaerenberg, 1964	11	11	9		2			
Vecchione *et al.*, 1964	16	16	14		2			
Pellet *et al.*, 1964	28	24	1	1	7	10		5
Kleinfeld *et al.*, 1966	21	21	11	1	1	8		
Hany *et al.*, 1967	8	8	1			5		2
Poggi & Carosi, 1968	17	14	1		8	2		3
Total	361	305	126	15	70	67	9	18

[a] The following criteria were used for classification:
Restrictive profile: RV and total lung capacity below 90% of expected value, and $FEV_1 \geq 70\%$ of FVC.
Obstructive profile: RV 110% or more above expected value, and FEV_1 less than 70% of FVC.
Alveolo-capillary block: RV and FEV_1 within 10% of expected value; and evidence of impaired gas diffusion, from either reduction in D_L, reduced arterial O_2 saturation (SaO_2) or increased A–a O_2 tension differences (Marks *et al.*, 1957).

DESCRIPTIVE STUDIES OF LUNG FUNCTION IN ESTABLISHED ASBESTOSIS

Asbestosis, a disease characterised by a diffuse interstitial fibrosis, is generally thought to be associated with the restrictive and "alveolar capillary block" profiles of pulmonary function (Austrian *et al.*, 1951; Bader *et al.*, 1961, 1970; Baldwin *et al.*, 1949; Thomson *et al.*, 1965; Williams *et al.*, 1960; Wright, 1955). Most workers would be in agreement with the text book view of Tepper & Radford that "the physiologic changes are primarily those associated with impaired gas exchange and stiffening of the lung parenchyma" and that "diffuse obstructive emphysema, so commonly seen in silicosis, is not apparent in asbestosis" (Gregoire, quoted by Smith, 1956).

However, several recent reports (Fournier-Massey *et al.*, 1971; Muldoon & Warwick, 1972; Pilat *et al.*, 1971) express the view that an obstructive function profile is not uncommon in the presence of asbestosis. This led Fournier-Massey (1973) to make a critical review of the published data, based on 305 individual case reports (Table 3). From this analysis, it is clear that asbestosis is associated with a variety of lung function profiles, involving not only those tests thought to be dependent primarily on the state of the lung parenchyma (e.g., lung volumes and gas exchange) but also those thought to be primarily dependent on the state of the airways (e.g., forced expiratory flow rates). If it is correct that most lung function tests are polyvalent, i.e., are dependent on the state of both the airways and of the parenchyma (Mead, 1971), then the obstructive profile in asbestosis may be due to secondary effects of the interstitial disease process on the airways. An alternative possibility is that it reflects primary effects on the airways themselves and may or may not be causally related to asbestos exposure. It remains to be determined whether or not the obstructive profile is more common in asbestos-exposed individuals than in persons not so exposed, after standardising for such things as smoking, urban residence and dust (as opposed to fibre), and if so whether there is a dose relationship.

Following Mead's perceptive analysis (1971), one can conclude that the more polyvalent a test the more valuable it will be in health surveillance or early detection, particularly if the health hazard (in this case, asbestos dust exposure) affects both airways and parenchyma, as Table 3 above would seem to indicate. There is empirical support for this line of reasoning in our own finding (Becklake *et al.*, 1972) that VC or IC appear to be the tests most sensitive to low dust doses in the Quebec asbestos-exposed workers, and in a study by Thomson *et al.* (1965) which used the technique of discriminant analysis to distinguish "asbestosis" cases in exposed but non-certified individuals. In a later study, this group (Regan *et al.*, 1971) used a principal component analysis on the data obtained from a field study of 201 asbestos-exposed workers; from this they concluded that decreases in T_L (diffusing capacity) and VC were the most useful criteria for measuring the severity of the disease, but that these tests could rarely distinguish between asbestosis and airways disease.

SUMMARY

The biological effects of asbestos exposure on lung function may be measured by a variety of techniques depending on the purpose for which the measurements are to be made.

If the purpose is to study an asbestos-exposed population with a view to defining whether or not a health hazard exists, it is appropriate to measure VC or IC alone or together with RV, and D_{LCO} (SB) (transfer factor).

If the purpose is health-surveillance, VC, IC or FVC appear to be the most satisfactory measurements; although it is likely (and remains to be tested) that measurements reflecting the state of the small airways will be most sensitive to the earliest effects of asbestos dust exposure.

If the purpose is descriptive, i.e., to document the patho-physiological changes which characterise asbestosis in its various stages, any or all of the many available tests for the study of overall or regional lung function may be used.

If the purpose is to evaluate the effect of pulmonary malfunction due to asbestos exposure on the overall function of the individual, a detailed study of adaptation to progressively increasing exercise loads is called for:

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Discussion summary

D. C. F. MUIR [1]

Clinical examination, radiology and lung function studies were clearly recognised during the discussion as being different approaches to the same problem, and that the aim of each was to detect and quantify possible effects of an environmental hazard on an exposed population. All results obtained with these methods must, eventually, be correlated with the measured exposure; and the chairman stressed the importance of attempting to obtain more precise information about the previous exposure of workers now being examined. This was seen clearly to be difficult, but its limits had not been reached in most cases, and the information was too valuable to be lost. Not the least reason for this, as pointed out by one speaker, is that the exposure history is the only feature specific to the diagnosis of asbestosis in the individual subject.

The difficulty of obtaining reproducible results from clinical examination was now known to be a fact of medical life, and it did not cause surprise that this should emerge in a study of asbestosis. The possibility of recording chest sounds was discussed but was not felt to be a practicable measure on a large scale. Finger clubbing in particular was considered to be difficult to recognise in a working population, to be a late sign of the disease and, in common with all the other features discussed, not diagnostic of asbestosis in itself. Chest pain was not uncommon in established asbestosis but was also a late sign.

The availability of the standard films of the new ILO U/C 1971 classification of pneumoconiosis was announced, and these could be obtained from the ILO, Geneva. The classification was now extended to include asbestosis. The lack of a uniform radiological technique was noted, as was the lack of agreement about the features of good quality films. The desirability of including in the classification information about loss of normal vascular markings was stressed. High voltage techniques were noted to be gaining popularity in the clinical field since they improved the quality of the film, and this was particularly so in female subjects; however, these methods undoubtedly reduced the visibility of calcified plaques. Tomograms did not appear to be helpful in the diagnosis of asbestosis, although they might be of value in silicosis studies.

The value of individual physiological tests was discussed. There was a general feeling that the results of complicated measures such as the carbon monoxide transfer test were disappointing in the early stages of asbestosis and that serial tests of vital capacity were probably the best single measure. Such measurement had the great advantage that each worker acted as his own control. The age at which the vital capacity started to decline in the normal population is not known, and several groups are working on this problem. The possibility of transient physiological changes in relation to exposure during the working day was mentioned, but no information was available.

One speaker expressed disappointment at the lack of progress since the last meeting of the working group, which he felt was evident from the main papers. The essential aim, to devise means to detect damage before it was progressive, in the absence of further exposure, had not been reached. However, he did not describe methods whereby he thought progress should have been made.

The meeting attempted to answer the one question to which all participants clearly gave first importance, namely, "which is the most sensitive method for detecting the effects of asbestos exposure?" No single answer was forthcoming, and it was concluded that all available sources of in-

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formation should be employed for the benefit of the individual. There was a general feeling that the question should be reworded so that it was clear whether the information was required for epidemiological purposes or for other specific reasons such as the diagnosis of asbestosis in the individual.

ASSESSMENTS OF METHODS USED IN THE STUDIES OF THE BIOLOGICAL EFFECTS OF ASBESTOS. 2. PATHOLOGY

Chairman — H. Otto

Rapporteur — H. T. Planteydt

A trial of techniques for counting asbestos bodies in tissue

P. D. OLDHAM [1]

A variety of different methods is available for determining the number of asbestos bodies in lung tissue, but it is not known if these methods give similar results. Further, little is known of the variation in the number of bodies in relation to degree of exposure to asbestos, nor whether in the individual the number of bodies varies in different parts of the lung.

An experiment of limited scope was conducted in order to accumulate some numerical information concerning these factors. It was intended to cover the possible differences between two basically different methods: (*a*) counting of bodies seen in thin sections under the microscope; and (*b*) counting of bodies seen after digestion of a quantity of lung tissue and its redispersion; and to obtain some information about the variations of the asbestos body count from one part to another of the same lung.

Observers have strong preferences concerning the thickness of thin section and the different staining methods. It was decided therefore to let each observer use his own preferred technique. Any conspicuous differences in the resulting counts could be examined to establish whether they were due to variation among the observers or variation among the techniques.

STATISTICAL DESIGN

It was considered that the maximum number of specimens to be counted by each observer should be limited to about 30. In order to cover the four quarters of the lungs, with and without pleura, eight specimens would be needed; consequently four cases were chosen, two with asbestosis, and two without occupational exposure to asbestos but who lived in an area where about a quarter of post-mortem cases had been found to have asbestos bodies in the lungs. From each case the eight blocks were taken in pairs from the right upper, left upper, right lower and left lower zones, one of each pair including some of the pleural surface.

Five sections were cut from each block, three of 6 μm thickness and two of 30 μm. Of the three 6 μm sections, one was unstained, one was stained with haematoxylin and eosin, and the third with Perls's stain. One of the 30 μm sections was stained with Perls's stain and the other was left unstained. The sequence in which each block was cut was carefully randomised to ensure that variation in the profusion of asbestos bodies across the block fell randomly among the five sections and the residue to be used for digestion and redispersion. For example, in case 2, the block including pleural surface from the right lower lobe was cut as follows:

6 μm	Perls
6 μm	unstained
Residue	
30 μm	unstained
6 μm	H & E
30 μm	Perls

On each of the thin sections a square 1½ cm × 1½ cm was marked in ink, unless one dimension was

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too small, in which case a rectangle of equal area was marked. Observers were required to estimate the total number of bodies within the marked area.

OBSERVERS

Seven observers returned their counts in sufficient time for analysis. Table 1 shows their identifying letters, the type of section they examined, and notes on the technique they adopted. One additional observer who examined 30 μm unstained sections reported so many as "uncountable" that his results have had to be discarded.

Table 1

Observer	Type of section	Method
A	30 μm, unstained	As much section as was necessary to observe 100 bodies was scanned. If this was less than the whole, the number of fields scanned was recorded.
B	30 μm, unstained	When many bodies were seen, a range (e.g., 300–500) was quoted. The upper limit mentioned was 100 bodies; in other cases the report was "innumerable".
C	30 μm, unstained	A sample of fields was scanned, and the position of each body was recorded on a grid (method of C. H. Um). Fibres and pseudo-bodies were recorded separately.
D	30 μm, Perls	When the number of bodies was large, an estimate was given in thousands. The largest estimate given was 4000; in other cases the count was stated as "thousands".
E	6 μm, Perls	The exact number of bodies, fibres, and pseudo-bodies seen was given in every case.
F	6 μm, Perls	The exact number of bodies, fibres and pseudo-bodies seen was given in every case.
G	Residue of block	Digestion of tissue, redispersion of residue, and counting of bodies and fibres in Fuchs–Rosenthal chamber (method of C. Gold).
H	6 μm, H & E	Systematic search of marked area, or until several hundred bodies were seen, when number was scaled up by ratio of searched area to whole.

RESULTS

In order to make comparisons, the raw counts must be adjusted to some standard. How this should be done is debatable, but, as will be seen, the particular method adopted cannot materially have affected the conclusions: observer C expressed his results as the number of bodies per cubic millimetre, and his complex method of estimation made it difficult to express his results in any other form. Accordingly, all the other counts were expressed in these terms, using the assumption that the volume scanned on a 6 μm section was 1.35 mm^3, and that on a 30 μm section, 6.75 mm^3. It was pointed out by several observers that the actual areas marked on the sections were not always 2.25 cm^2, and were sometimes as small as 1.7 cm^2. Thus, errors of 30% or more are introduced by assuming a standard area. However, it will be seen that the range of disagreement among the observers was such as to make this unimportant.

Observer A made counts of 100 bodies in varying numbers of microscope fields; these were adjusted to the standard by making simple geometrical assumptions about the effective area of each field. Those sections said by observers B and D to show an uncountable number of bodies were arbitrarily given a count corresponding to the largest number per mm^3 counted in the series.

That these grossly simplified procedures were nevertheless adequate can be seen from Figure 1. The range of results is such that, here and throughout, logarithms of the counts are used. Figure 1 shows the counts in all 32 sections, one column to each section. The first eight columns relate to case 1, the next eight to case 2, and so on. Within each case the order of the sections is the same:

Right upper zone, with pleura
without
Right lower zone, with pleura
without
Left upper zone, with pleura
without
Left lower zone, with pleura
without

Zero counts have, arbitrarily, been plotted at 0.01 bodies per cubic millimetre. In case 2, left upper zone without pleura, and in case 3, right upper zone without pleura, the counts of observer F were pseudo-bodies; he specifically recorded no asbestos bodies. Since all the other observers saw asbestos bodies, it seemed best to give the number of objects F saw rather than zeros.

It will be seen that the range of counts on each section is more than tenfold, however it is clear

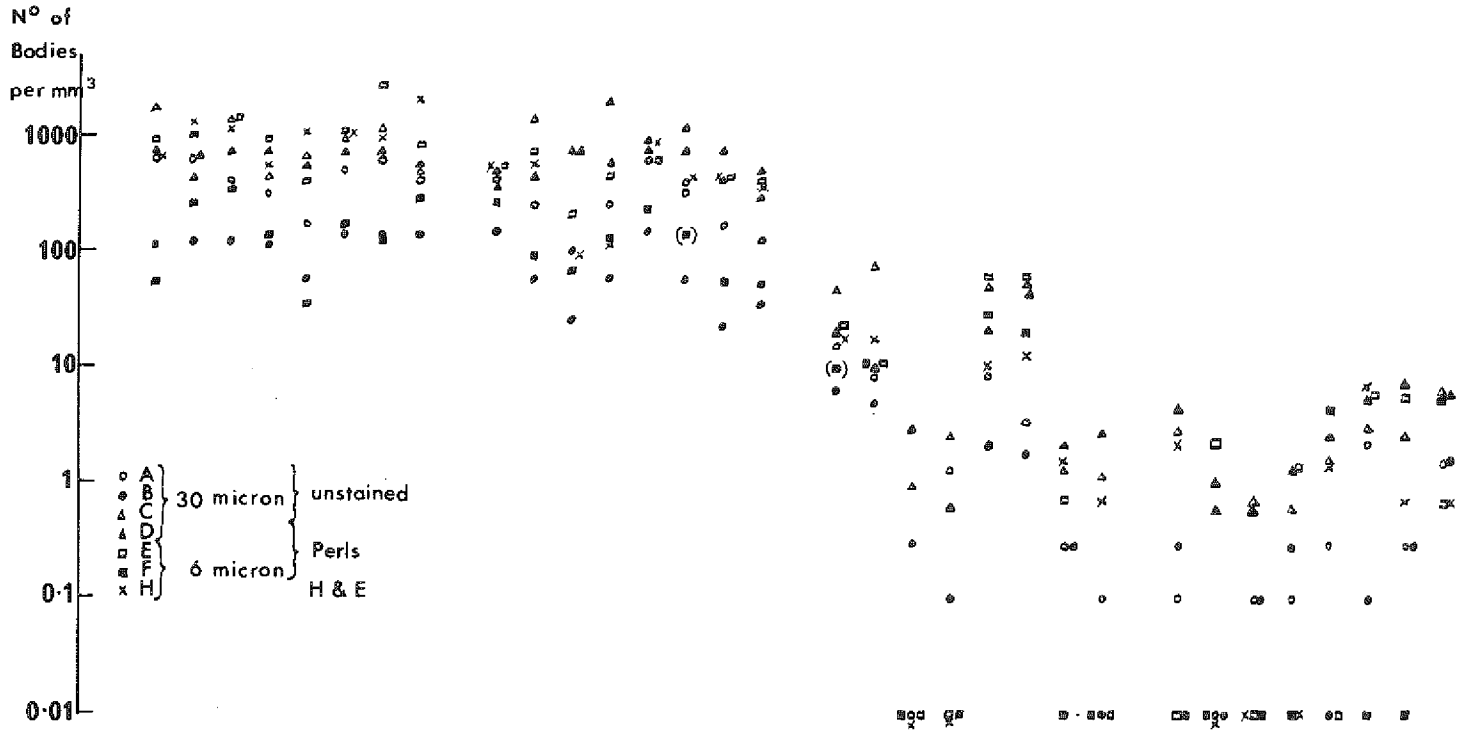

Fig. 1. Counts by seven observers on each of 32 sections. Each column is one section; successive sets of eight sections come from the same case.

that there is a degree of consistency in the results. No particular technique gives consistently high or low results; the variation seems attributable primarily to observers. Such distinction as there is among the four cases is made equally well by all observers and by all techniques.

There is no consistent difference between the pleural sections and the purely parenchymal ones. Only in case 3 is there a marked difference between the upper and lower parts of the lung: all observers counted more bodies in the four sections from the upper lobes. In case 4 four of the observers found more bodies in the left lung than in the right; three made counts in 6 μm sections, and the fourth was observer A who counted 30 μm unstained sections.

Figure 2 shows the results obtained by observer G, who examined the residue of each block by digestion too late to be included). It will be seen that the correlation is good. The sixteen points at the top right-hand side come from cases 1 and 2; the four samples from the upper lobes of case 3 appear at about 10 mm^3; while case 4 and the lower lobes of case 3 provide the remaining points. In three cases where observer G found no asbestos bodies (although he did find fibres) the points appear with downward arrows, indicating that the estimates are less than the 1.4 bodies per mg shown.

DISCUSSION

No detailed numerical analysis of these results is called for. It is evident however that, with all the

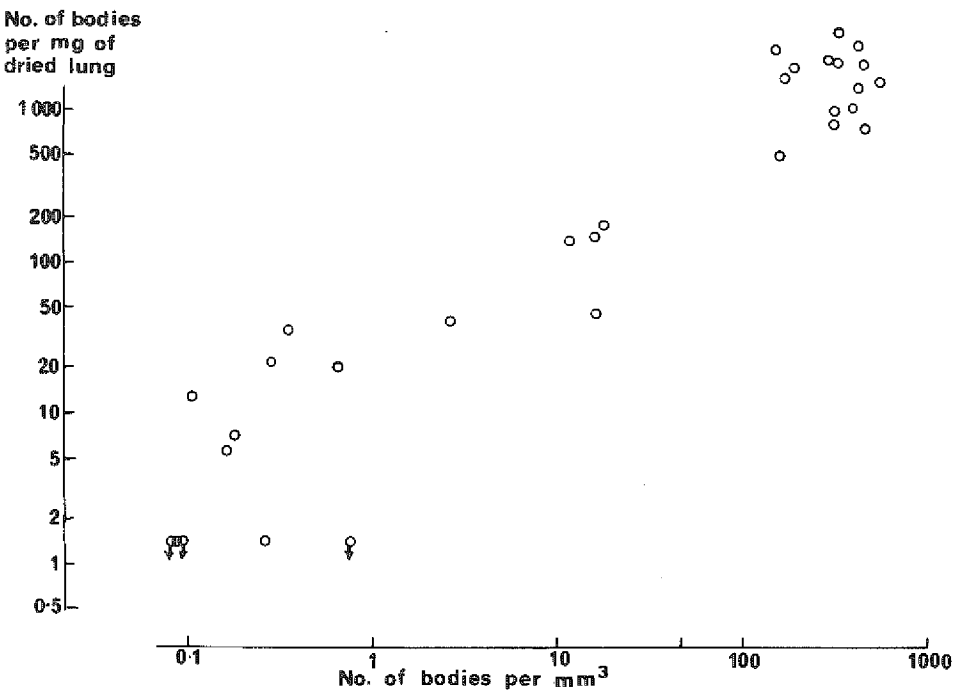

Fig. 2. Correlation between the counts by one observer of redispersed, digested blocks of lung and the average count of thin sections from the same blocks.

and redispersion. His results are expressed on a mass basis, as the estimated number of bodies per unit weight of dried lung tissue, and are plotted against the geometric mean counts per mm^2 of observers A to F (the results from observer H arrived

techniques used, there is a range of uncertainty of about an order of magnitude, and that this should be borne in mind when assessing reported numerical counts of asbestos bodies. At the same time, no one method appears to have a serious bias. Clearly

30 μm sections, stained or unstained, show embarrassingly large numbers in cases of asbestosis; thus it would be advisable to specify an exact procedure to be adopted in such cases. On the other hand, 6 μm sections are more likely to give zero counts in cases where other techniques can show asbestos bodies to be present; this may be undesirable in the study of diseases suspected to be asbestos-related.

The scrupulous but extremely laborious technique introduced by Um (1971) evades both these difficulties; however the results which it provides are not so markedly different from those obtained by simpler methods as to justify a recommendation for its general adoption.

Gold's technique (Gold, 1968) compares very well in numerical terms with thin-section methods, and it is evident that the digestion of lung tissue with KOH does not cause serious loss of asbestos bodies as had been feared.

It appears that practical considerations of expediency can dictate the choice of method. It is probable that material reductions in the differences between observers could be achieved, with any technique, by a set of specified rules for scanning under the microscope. This should probably involve a rectangular grid, a set procedure to relate this to a marked area on the slide, and conventions for sampling the section when large numbers of objects are seen.

The evidence provided by this study of variation in the profusion of asbestos bodies from one part of the lung to another is extremely limited; but it is at least clear that large differences may occur in some cases.

SUMMARY

Sections were cut from 32 blocks of lung tissue taken from four subjects. 6 μm and 30 μm sections were stained either with Perls's stain, or haematoxylin and eosin, or left unstained. They were scanned by eight different observers, and the profusion of asbestos bodies seen was recorded. The residue of each block was digested with KOH, redispersed, and then scanned.

A tenfold variation was found in the counts obtained; this appeared to be more a function of difference in observer than in technique. Nevertheless, the tissue samples from the four cases were distinguishable one from another; some material differences between the upper and lower lobes of one case were seen; and the average count of asbestos bodies in the thin sections correlated well with the count in the redispersed digested residue.

ACKNOWLEDGEMENTS

This work was carried out by the following pathologists of the UICC Committee on Asbestos and Cancer: Dr G. Chiappino; Dr R. Guy; Dr M. Kannerstein; Prof D. Magner; Dr L. Meurman; Prof H. Otto; Dr H. T. Planteydt; Prof L. Santi; Dr J. C. Wagner; Prof I. Webster; co-opted, Dr C. Gold and Dr C. H. Um.

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Methods for assessing asbestos fibres and asbestos bodies in tissue by electron microscopy

F. D. POOLEY [1]

Since the majority of dust particles formed by the commercial asbestos minerals are submicroscopic, the electron microscope is the only practical instrument suitable for assessing the asbestos fibre content of tissues. Asbestos bodies, because of their larger dimensions, are readily discernible with a light microscope, and their presence can always be enhanced by staining the iron-rich material of which they are composed; however, their fibre cores can be identified only by using an electron microscope.

DIRECT EXAMINATION OF TISSUE SPECIMENS

Specimens can be examined directly in the electron microscope if the tissue can be cut into suitably thin sections with a microtome. The normal thickness of electron microscope specimens is of the order of 100–600 nanometres (nm). In the normal electron microscope, sections thicker than 600 nm rapidly become opaque, so that details of particulate inclusions and tissue morphology become obscured.

Because electron microscope sections must be very thin their preparation is very difficult, and this is especially so when the tissue contains mineral particulate matter with dimensions larger than the section thickness. These large particles are usually torn from the section, causing a great deal of damage in the process. If, however, the mineral particles can be cut easily by the microtome blade, the specimens which can then be prepared give the maximum amount of information on the association of the dusts with the tissue.

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Of the asbestos minerals only chrysotile asbestos fibres are relatively easily sectioned, as shown by Yada (1967). The amphibole fibres, crocidolite, amosite and anthophyllite, are harder and very difficult to cut. Suzuki & Churg (1969) have reported the successful sectioning of tissue containing chrysotile; Davis (1964a, b) has also employed thin sections in his studies of asbestos bodies in animals and humans. This technique has, however, found little application in the general study of the presence of asbestos and asbestos bodies in tissue.

One promising application of the method, however, is in the use of thicker tissue sections, of the order of 6–10 μm, in high voltage electron microscopes, which have a penetrating power far in excess of that of normal ones; and also in the latest electron microscope models, which have scanning and transmission facilities enabling thicker sections to be studied.

DIGESTIVE PROCEDURES

As direct examination of tissue containing asbestos fibres presents great difficulties, some form of extraction process must be employed. Tissues can be broken down by acid, alkaline and enzyme solutions, thus releasing their mineral particles, and these can then be concentrated for subsequent observation. Gold (1967) formulated a procedure for releasing asbestos fibre in which diced pieces of tissue were dissolved in a hot 40% KOH solution. This procedure has been employed extensively in the analysis of lung tissue for asbestos fibre, and Langer *et al.* (1971) have reported data obtained using this technique. The technique involves a number of preparative steps of which the most severe

is the washing and centrifugation of the tissue residue in order to remove the alkaline solution. As this washing must be performed several times, there is the possibility of loss of particulate material. This loss of material reduces the usefulness of the technique as a quantitative procedure; however, providing that the loss of material is random in nature and the percentage reduction is fairly constant, the process is extremely useful for comparing the fibre content of tissues, and also for the qualitative assessment of their fibre content.

Hot KOH does not dissolve all the organic material contained in lung tissue specimens. Often large quantities of undissolved tissue remain in suspension; and the residue can also be heavily contaminated by carbonaceous and other mineral particles whose presence can make the detection and assessment of asbestos fibre in the sample a very hazardous operation. To reduce this level of contamination, alkaline-digested residues are often ashed.

Other digestive procedures, using formamide and enzyme solutions, also produce residues in which the tissue is not always entirely dissolved, and which also require subsequent treatment. Gross *et al.* (1968) describe a method for isolating ferruginous bodies and fibres using sodium hypochlorite followed by several washings, first in chloroform and ethyl alcohol, and then in water, before obtaining a residue. The choice of a tissue solvent, therefore, often depends upon the research group involved and the confidence they have in any particular procedure.

The quality of the tissue extract produced by any technique will be influenced by a number of factors, among which we can include:

(*a*) the choice of tissue solvent;
(*b*) whether the tissue is fresh or fixed;
(*c*) whether the tissue is diseased or normal; and
(*d*) the quantity of retained particulate material, both organic and inorganic, contained within the specimen.

Whichever digestive solution is chosen for use, however, it should not be highly acidic, since it is known that chrysotile fibre can be severely affected by acid solutions.

Having produced an extract deemed suitable for examination, it is then necessary to present this material to the electron microscope for detailed analysis of fibre type and content. The difficulty of obtaining quantitative information using this technique lies in assessing from what quantity of the initial tissue the electron microscope specimen has been extracted. Also, great care must be taken to ensure that none of the small aliquot of residue applied to the specimen grid (which is generally only 3 mm in diameter) is lost in the transference procedures. These difficulties do not apply to the optical examination of digested tissue residues using a Fuchs–Rosenthal chamber, where relatively large quantities of material contained within a known volume can be handled.

Any digestive procedure can be described as a bulk extraction technique, and this can give information on the fibre content of only a known volume of lung tissue. Considering the difficulties which are involved in preparing such specimens for examination in the electron microscope, it may be concluded that digestive procedures are excellent for qualitative work but suitable for deriving only relative quantitative information.

ASHING PROCEDURES

Tissue can be prepared either by a bulk ashing procedure or by the ashing of thin sections of tissue. Ashing of large quantities of macerated tissue has been found to be unsuitable for electron microscope work because of the large quantity of tissue ash remaining in the sample. This ash has a tendency to bind particles into aggregates and can be removed only by acid washing, which is detrimental both to asbestos bodies and to chrysotile fibres. The incineration of thin sections of tissue, 6–30 nm in thickness, on their glass slides involves only a small amount of material; and the ashed residue also retains histological data. The distribution of fibres and bodies remains as in the original unashed section, and some tissue ash remains to give an outline of the original anatomy. The quantity of tissue ash remaining after incineration of a section depends on the initial thickness of the section and also on the extent of microincineration. It is often advantageous to prevent the full ashing of the tissue so that a partly carbonised outline of the original tissue is retained.

Ashing of tissue should be performed at as low a temperature as possible to prevent structural changes in the asbestos fibres present, especially of chrysotile. The use of low-temperature ashing equipment is not entirely justified, since the tempera-

ture at which the tissue is finally reduced depends a great deal on the quantity of combustible material present in the specimen which reacts exothermically to produce localised high temperature areas during ashing. Pooley *et al.* (1970) have shown in lung tissue that chrysotile is visibly unaffected when the tissue is ashed at 500°C for periods of 10–15 minutes.

Specimens of bulk ashed tissue are prepared for examination in the electron microscope by sampling from a suspension of the ash in either distilled water or alcohol, as with digested tissue residues. The difficulties involved in the preparation and interpretation of bulk ashed samples are therefore similar to those for digested residues. The preparation of ashed thin sections is a little more complicated, but a procedure has been outlined by Pooley (1972) which involves the use of a thin film of water-soluble plastic for the transfer of the ashed residue from the microscope slide to the electron microscope specimen grid.

Bulk ashing of tissue to release asbestos fibre can be considered useful for qualitative determinations of asbestos fibre; but again, as with digested tissue extracts, the technique is, perhaps, suitable only for relative quantitative work. The same criticism can be applied to the examination of ashed tissue sections, where some random loss of material is to be expected in the preparation of the electron microscope specimen. Microincineration can be considered useful, therefore, for locating particles in specific tissue areas and for qualitative and semi-quantitative determinations of the asbestos fibre content.

DISCUSSION

When deciding what method should be adopted for the examination of tissue for asbestos fibre content, using the electron microscope, the aims of the investigation should be clearly stated so that a suitable technique can be adopted. The method should allow the presentation of a representative fraction of the fibres contained in the tissue. It should allow the removal of as much tissue, or other material, likely to interfere with the observation and detection of fibre. The method should preserve as much as possible the character of the fibre dust particles held in the tissue. The technique should be simple, with few preparative steps, to prevent excessive or selective loss of fibres. In the preparation of thin tissue sections, when several paraffin blocks are to be cut one after the other in the microtome, care should be taken to prevent contamination of the blocks and of the cut sections. Contamination can also occur in the preparation of digested residues, as stated by Langer *et al.* (1971).

Asbestos fibres are relatively inert and are not harmed to any great extent by ashing or by digestive techniques. Formations of asbestos bodies are, however, more fragile and their composition and structure can be changed by heating and digestive procedures. Thus, they are best studied *in situ*, if possible. Bulk extraction by ashing or digestion gives no information concerning tissue-fibre association, while microincineration does; on the other hand, bulk extraction techniques can concentrate fibre for examination purposes.

It would appear that there is no one simple method available for presenting material to the electron microscope in a form suitable for obtaining all the necessary information in investigations of asbestos fibre association with tissue. Where possible, therefore, several methods should be applied.

SUMMARY

At the present time no single method is available for preparing tissues for electron microscopy so that all the relevant information concerning their asbestos fibre and asbestos body content can be elicited. A combination of methods must be employed. Most techniques are suitable for qualitative appraisal of tissue specimens, but the large number of steps normally employed in preparing a specimen for the electron microscope renders them only relatively useful for quantitative work. The author has found that a suitable combination of methods for preparation of a tissue involves KOH digestion and an examination of ashed thin sections.

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Criteria for the diagnosis and grading of asbestosis

K. F. W. HINSON,[1] H. OTTO,[2] I. WEBSTER [3] & C. E. ROSSITER [4]

The grading of the severity of pulmonary fibrosis in autopsy and lung biopsy material should be attempted so that changes may be correlated with respiratory tests, radiological findings, and the nature, intensity and duration of exposure to respiratory hazards.

Our proposal is an extension and rationalisation of the grading recommended by the Working Group on Asbestos and Cancer (UICC, 1965), so that it may be applied to many cases of pulmonary fibrosis, including asbestosis. The severity of other lesions as seen on microscopic examination would have to be standardised. Primarily, the classification is descriptive only, the decision as to the cause of the pulmonary fibrosis being based on other information.

The Working Group on Asbestos and Cancer, 1965 recommended that, for asbestosis, an attempt at classification should be made not only by assessing the severity of fibrosis macroscopically, but also by an assessment of the extent of changes in the lungs. Its preliminary suggestion was as follows:

Extent of lung involvement		*Degree of asbestosis*		*Degree of interstitial fibrosis*
Slight	→	Slight	←	Slight
Moderate	→	Moderate	←	Moderate
Marked	→	Marked	←	Marked

It will be noted that in the "slight" and "moderate" categories this scheme places more emphasis on the importance of the extent of the disease than on the microscopic findings.

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METHODS

At necropsy, the parietal pleura should be removed together with the other thoracic contents. It is strongly recommended that at least one lung should be distended with fixative before sectioning, otherwise assessment of the extent of fibrosis and of emphysema will be difficult. At autopsy, note should be made of the size and distribution of all pleural lesions. (Plaques are defined as localised, stiff, horn-like, fibrous thickenings which may be calcified, although earlier localised areas of fibrosis may also be present.) The mediastinal structures should be examined for the presence of neoplasm or tuberculosis.

After fixation the lungs should be sliced, and the presence of tumour, tuberculosis, bronchiectasis, bronchiolectasis or massive fibrosis should be recorded.

The origins of the blocks taken for microscopy must be left to the discretion of the pathologist, but if possible at least one should be taken from each lobe, only one of which should include the pleura. This will make possible an overall microscopic assessment of the degree of fibrosis.

Further blocks should be taken from the hilar lymph nodes and, in fact, from any abnormal areas suggestive of tuberculosis or neoplasm.

Tissue should also be preserved, ideally both fixed and frozen, for the demonstration of bodies and fibres in doubtful cases of asbestosis or suspected mesotheliomas, both for research and possible medico-legal purposes.

Sections should be stained routinely for examination of connective tissue.

CLASSIFICATION

The classification of pulmonary fibrosis is based both on the extent of lung involvement and on the severity of the fibrosis.

Extent

The extent of lung involvement by pulmonary fibrosis should be assessed by a combination of inspection and palpation. (The weight of the lungs may be of assistance in the absence of other pathological changes.)

In early cases, fibrosis is more easily appreciated by touch; in suspected asbestosis particular attention should be paid to the subpleural zones of the lower lobes. Webster has drawn attention to a "chronic pleurisy" which may affect those exposed to asbestos, which may lead to over-estimation of both the extent and severity of asbestosis, particularly when the grade is slight. This chronic pleurisy presents itself as a diffuse fibrous thickening which makes the pleura opaque over the lateral basal and apical segments of the lower lobes.

It is suggested that three positive grades of extent of fibrosis be recorded, recognising however that this may be difficult and that overlap will occur. The proposed assessments are:

A None.
B Where less than 25% of the lung substance is involved.
C Where 25–50% of the lung is affected.
D Where more than 50% of the lung is diseased.

Severity of asbestosis

This is assessed by microscopic examination of the sections. Differentiation among the grades is made by comparison with standard slides. The grades of severity are:

0 None.
1 The lesions consist of a slight focal fibrosis around respiratory bronchioles, associated with the presence of asbestos bodies.
2 The lesions are confined to respiratory bronchioles of scattered acini. Fibrosis extends to alveolar ducts and atria as well as to the walls of adjacent air spaces.
3 There is a further increase and condensation of the peribronchiolar fibrosis with early widespread interstitial fibrosis.
4 Few alveoli are recognisable in the widespread diffuse fibrosis; bronchioli are distorted.

(*Note.* Grade 1 is similar to the 1965 Working Group proposal for a minimal category.)

Other features

In the classification symbols are provided to note the presence of certain other changes:

bl	bronchiolectasis
br	bronchiectasis
ca	tumour
mf	massive fibrosis
pq	pleural plaque
pt	pleural thickening
tb	tuberculosis

Comments may also be made to elaborate the symbols or to add any other information not covered by the symbols.

DISCUSSION

The classification is purely a descriptive one, for degrees of pulmonary fibrosis, and no diagnosis of any particular cause is involved. Thus it is directly comparable with respiratory tests which assess changes independent of their causes, and also with radiographic examinations, where the ILO classification is used to quantify the extent of radiographic changes, also without considering cause. Thus pulmonary fibrosis, respiratory physiology and radiography can be interrelated.

A diagnosis of a particular cause can be made by using the results of the classification together with concomitant information. For example, if the pulmonary fibrosis classification is C3 (grade 3 severity for between 25% and 50% of the lung) and numerous asbestos bodies are present, then asbestosis may reasonably be diagnosed.

However, we make no proposals as to the grades of fibrosis which are compensatable, even if the nature of the disease is known, nor as to the requirements for other information needed to make a diagnosis. These decisions can be made only by the national compensation boards and by individual pathologists. Rather, the classification provides a means of standardising the assessment of pulmonary fibrosis so that such decisions can be made, and so that international and regional comparisons will be meaningful.

Figure 1 illustrates the proposals for the grading of fibrosis. It shows that we expect certain grades to be non-existent, e.g., those with apparent macroscopic involvement but with no fibrosis microscopically.

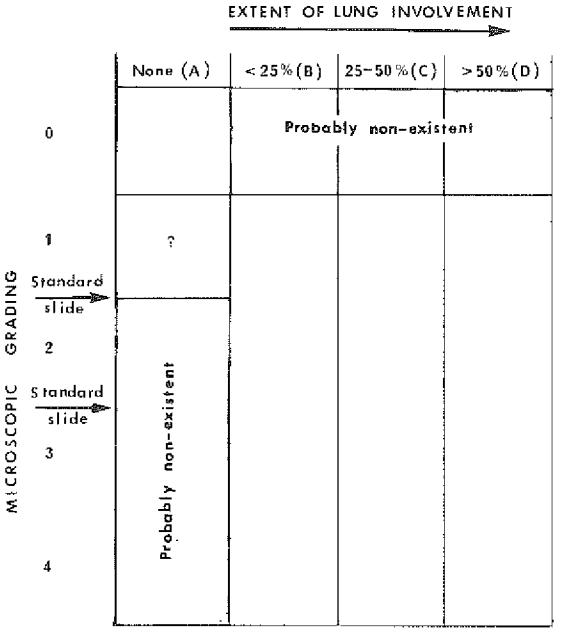

Fig. 1. Proposal for the grading of fibrosis.

Considering the particular case of asbestosis, we suggest that the presence of fibrosis with included asbestos bodies or fibres should be essential for positive diagnosis. However, it must be recognised that the severity of the fibrosis and the number of asbestos bodies are not always proportional (Gough, 1965). Problems in interpretation and assessment will also occur if the asbestosis has been associated with long-standing bronchial obstruction, bronchiectasis or chronic infection. It may also be impossible to distinguish the effects of previous or contemporary inhalation of other fibrogenic dusts.

Diffuse fibrosing alveolitis (Scadding & Hinson, 1967) in its cryptogenic form must also be considered; this leads to fibrotic destruction of the lungs leaving

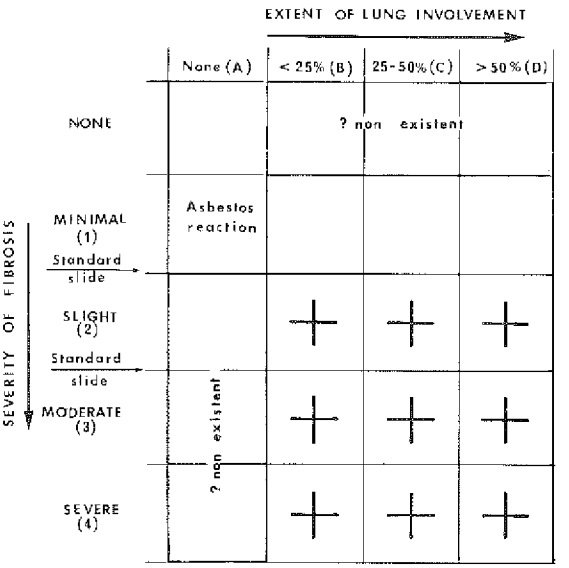

Fig. 2. Suggested modification of the proposal for the grading of fibrosis for application to asbestosis.

a honeycomb appearance, particularly in the lower zones. Should occasional asbestos bodies be found within the interstitial fibrosis, differentiation between the two conditions may be difficult. However, in lungs destroyed by asbestosis there are usually numerous bodies present in such bronchioles as persist.

Figure 2 shows those grades of fibrosis which might be said to indicate positive asbestosis when asbestos bodies are present; however, different countries or different situations may require different decisions.

SUMMARY

A proposal is made for the grading of pulmonary fibrosis, based on the extent of lung involvement and on the severity of the fibrosis. No diagnosis of any particular cause is involved, thus the grading may be compared directly with the results of respiratory and radiographic tests.

It is proposed that a necessary criterion for the diagnosis of asbestosis be the presence of fibrosis with included asbestos bodies or fibres. However, it is recognised that while the grading of pulmonary fibrosis would thus become internationally comparable, the diagnosis of asbestosis may still require different decisions for different circumstances.

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Diffuse mesotheliomas: observer variation in histological diagnosis

W. T. E. McCAUGHEY [1] & P. D. OLDHAM [2]

Assessment of histological material derived from suspected mesotheliomas has been carried out by a panel of observers since 1964. Their results, the names of the observers who examine the material and the final diagnosis are noted in a series of record books. The panel operates with the following rules, that (*a*) at least two members must see each case; and (*b*) if they disagree, a third member then sees it.

An analysis was made of this rather limited information. It soon became clear that some members of the panel were being called on to take part in the process more often than others. The proportion of all cases seen by the nine members who had from time to time taken part are given in Table 1. It will be seen that member A saw three cases in every four, observers B and C one in two, observer D one in four, while the remainder seldom took part.

Table 1. Proportion of cases seen by members of a mesothelioma panel

Panel member	Percentage of cases seen
A	73
B	51
C	52
D	28
E	1
F	4.5
G	4.0
H	0.3
J	0.3

Evidently, if judgements are biased, the overall level of diagnosis must be variable. In these records it is evident that when three or more members saw a case, the first two must have disagreed; however it is not possible to deduce which of them agreed with the third member. Among those members who saw numerous cases it is possible to tabulate the frequency with which they disagreed. Table 2 thus shows that observers A, B and D disagreed with each other in about 18% of cases; observer C disagreed with A in about 12% of cases, with B in about 14% of cases, but with D in almost a third of cases.

Table 2. Disagreements among those members of the panel who saw substantial numbers of cases

First two assessors	Number of cases sent to a third assessor/total no.	% of disagreements
A B	17/92	18
A C	11/93	12
A D	7/41	17
B C	6/43	14
B D	2/11	18
C D	7/22	32

There may be at least two distinct causes of such disagreement. Firstly, that observers were applying different criteria for diagnosis; and secondly, that there were differences in the interpretation of structural patterns in tumours.

In order to explore these possibilities a formal system was drawn up for recording microscopic details, as well as diagnostic opinion, for use with a small group of slides. The microscopic detail listed was of a type which might particularly relate to the assessment of a possible mesothelial tumour. The questionnaire is reproduced as an appendix to this paper.

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In the first investigation using the questionnaire 30 sections were circulated to three observers. All sections were from pleural tumour and were selected to cover as far as possible the wide range of tissue and cytological patterns which have been attributed to mesotheliomas. Several examples of metastatic tumour were also included. Completed questionnaires were obtained for 27 of the sections. The results were examined and agreement was found to fall into four classes:

(A) complete agreement that case was positive 7
(B) complete agreement that case was negative 6
(C) complete uncertainty about the diagnosis 9
(D) gross disagreement 5

Subsequently, three further observers examined the sections, and while their results somewhat blurred the distinctions among the four classes, the general conclusions remained the same. The analysis below is based largely on the readings of the first three observers.

Question (4) of the questionnaire gives the diagnostic categories:

0 definitely not mesothelioma
1 possible but unlikely mesothelioma
2 could equally well be either mesothelioma or metastic tumour
3 probably mesothelioma
4 definite mesothelioma

Class (A) above (complete agreement on positivity) contained those cases in which all three observers gave 3 or 4 as the diagnostic category. Despite this, answers to the detailed questions revealed substantial disagreements in almost every instance. There was hardly one question to which all the observers consistently gave the same answer. This was true of the main questions (1), (2) and (3) as well as for the conditional questions within them.

Class (B) (complete agreement on negativity) contained those cases in which all the observers gave 0 or 1 as the diagnostic category. In every case the observers agreed that the answers to questions (2) and (3) were negative (in class (A) there was one case in which this also was true). Disagreement on the questions within section (1) was extensive.

Class (D) (gross disagreement) was made up entirely of cases in which the same observer gave a definite negative while the other two gave a diagnostic category of 3 or 4. The second set of observers consistently joined the original two in giving probable or definite positive diagnoses. Thus, these cases really belong in class (A), and in discussion with the aberrant observer it became clear that his criteria for the diagnosis of mesothelioma and his analysis of histological detail differ considerably from those of the other observers. It should be remarked that this observer had not been a member of the panel.

Class (C) contained those nine cases in which all the observers, or two out of three of them, used the diagnostic category 2 (equal likelihood of a mesothelioma or of a metastic tumour). Agreement on the individual questions was, to a noticeable extent, better than in the classes where the diagnosis was certain. The readings of the subsequent observers in general confirmed this pattern, but there was a tendency for them to give one or other of the extreme categories, so turning this class into one of gross disagreement rather than of complete uncertainty.

Following examination of the questionnaires, they were reviewed at a meeting of the observers. This meeting further emphasised the marked differences in interpretation of histological detail in the tumours, and it also showed that some histological terms used were applied in different ways by the participating pathologists. That such factors were not necessarily paramount in explaining the disagreement, however, is illustrated by the fact that observers frequently agreed on the diagnosis and on which factors seemed most important to them, but they had given conflicting answers to the question about that factor.

In summary, this study has shown that the histological diagnosis of mesothelial tumours by a group of experienced observers is highly subjective. It is clear that considerably more systematic studies are needed to conclude how observers weight the various uncertainties involved in the diagnosis of mesotheliomas.

One can however end with the comment that, despite these uncertainties and despite the disagreements in detail, of the 27 cases in this trial there were 13 in which the diagnosis was agreed, 9 in which the diagnosis was agreed to be impossible, and 5 in which the disagreement was caused by a single observer who was not regularly assessing suspected cases. The implications for the work of the mesothelioma panel are not discouraging.

APPENDIX

OBSERVER VARIATION STUDY ON MESOTHELIOMAS

Slide No.: *Observer:* *Date:*

(1) Does the tumour contain an epithelial-like component? ..... Yes ☐ No ☐

If so, does this component show any of the following features:

(a) a tubular pattern ..... Yes ☐ No ☐

(b) a papillary pattern ..... Yes ☐ No ☐

(c) a tubulo-papillary pattern ..... Yes ☐ No ☐

(d) sheets or nests of undifferentiated but regular spheroidal or polyhedral cells ..... Yes ☐ No ☐

(e) sheets or nests of undifferentiated pleomorphic cells ..... Yes ☐ No ☐

(f) if the answer to (a), (b) or (c) is "yes", are the lining cells mainly ..... flattened ☐ cuboidal ☐ columnar ☐

(g) if the answer to (a), (b) or (c) is "yes", is there conspicuous cellular uniformity? .... Yes ☐ No ☐

(h) do the nuclei of epithelial-like tumour cells have a conspicuous vesicular quality? .. Yes ☐ No ☐

(i) are nucleoli prominent in the epithelial-like tumour cells? ..... Yes ☐ No ☐

(j) are mitoses numerous? ..... Yes ☐ No ☐

(k) is there any evidence of secretory activity by the tumour cells, e.g., mucus? ..... Yes ☐ No ☐

(l) is the amount of stroma associated with the epithelial-like component ..... small ☐ moderate ☐ plentiful ☐

(m) is the stroma cellular? ..... Yes ☐ No ☐

(2) Does the tumour contain a sarcoma-like component? ..... Yes ☐ No ☐

If so, does this component show any of the following features?

(a) numerous spindle or fusiform-shaped cells ..... Yes ☐ No ☐

(b) bundles or bands of spindle or fusiform-shaped cells ..... Yes ☐ No ☐

(c) predominance of non-spindle shaped cells ..... Yes ☐ No ☐

(d) is the amount of matrix around the sarcoma-like cells ..... small ☐ moderate ☐ plentiful ☐

(e) does the matrix show a prominent hyaline character? ..... Yes ☐ No ☐

(f) does the stroma in the tumour take up any unusual patterns? ..... Yes ☐ No ☐

(3) Does the tumour have a mixed pattern? ..... Yes ☐ No ☐

If so,

(a) does the epithelial element predominate? ..... Yes ☐ No ☐

(b) does the sarcomatous element predominate? ..... Yes ☐ No ☐

(c) if both epithelial and sarcomatous elements are present, are they mixed, at least in part? ..... Yes ☐ No ☐

(4) Diagnostic category 0 1 2 3 4

(0 = definitely not mesothelioma, 1 = possible but unlikely mesothelioma, 2 = could equally well be either mesothelioma or metastatic tumour, 3 = probably mesothelioma, 4 = definite mesothelioma)

(5) Are there any diagnostically significant features of the growth which have not been indicated? ..... Yes ☐ No ☐

If so list below:

(a) .....

(b) .....

(c) .....

(d) .....

(6) If you feel that the tumour is not a mesothelioma what is the most likely source of the growth? .....

Factors in diagnosis (list here in order of importance, the numbers of the six factors, e.g., (1a), (2f), noted above which most influenced you in assigning a diagnostic category):

(A) (B) (C)

(D) (E) (F)

Comment:

SUMMARY

The problems involved in the diagnosis of mesotheliomas of the pleura, which may lead to disagreements among observers, have been explored by asking observers to record systematically the features seen in histological sections. Considerable differences in the interpretation of histological detail were revealed, as were differences in the usage of histological terms. Further systematic studies of this type are called for.

Histochemical studies in the diagnosis of mesothelioma

M. KANNERSTEIN,[1] J. CHURG [2] & D. MAGNER [3]

The histologic variation of mesothelioma requires the use of all available information to increase the certainty of diagnosis. The capacity of many mesotheliomas to produce the acid mucosubstance, hyaluronic acid, has frequently been investigated for its possible diagnostic value. Among those who have used and discussed this property are Winslow & Taylor (1960), Wagner *et al.* (1962), Churg *et al.* (1965) and recently Oels *et al.* (1971). In most of these reports the results are not conclusive as to the actual utility in routine diagnosis. Demonstration of the lack of neutral mucosubstance, for assistance in excluding carcinoma, has found more acceptance as a relatively routine method.

We have attempted to assess the diagnostic usefulness of the properties referred to above, studying a relatively large series of mesotheliomas.

METHODS AND MATERIALS

To conform to our purpose of determining the desirability of methods for routine use, we limited staining techniques to the periodic acid-Schiff reaction, according to McManus (1948), after exposure of the tissue sections to diastase (DPAS), and to the Hale colloidal iron stain as modified by Rinehart & Abul-Haj (1951), before (CI) and after the use of testicular hyaluronidase (HCI). Comparison of CI- and HCI-stained preparations indicates the extent to which any acid mucosubstance present consists of hyaluronic acid.

In 70 cases taken from a group of over 200 diagnosed as mesothelioma, pleural and peritoneal, we have reviewed sections stained by the methods indicated. In keeping with our general principles for the evaluation of certainty of diagnosis, we divided the 200 cases into three categories: definite, probable and possible.

Cases in the definite category corresponded to the well-defined histologic patterns as described by numerous authors, including McCaughey (1965) and Churg *et al.* (1965), with consistent clinical and gross morphologic data. Those in the probable group approached these criteria, with one or more elements lacking or discrepant; and the possible group included those with non-specific, atypical appearances or those cases accompanied by detracting clinical or gross pathological features. The 70 cases used in this study came from within each group in random fashion.

We have, in addition, for purposes of comparison, included 26 cases diagnosed as carcinoma. Fourteen of these were adenocarcinomas, 7 arising in the lung. Others were solid carcinomas, 4 being of pulmonary origin, and the remainder from a variety of sources.

The material was fixed in formalin, except for a few cases in Bouin's solution.

RESULTS

As will be seen in Table 1, 64 of 70 mesotheliomas showed no reactivity to DPAS. Six cases presented a minimal or questionable reactivity. We are not referring to the fine granularity described by Fisher & Hellstrom (1960), which we found present in varying degrees in a number of mesotheliomas, but which we could not relate diagnostically. In the cases listed here there was considerable granular fuchsinophilic colouration of occasional cells, and/or a rare vacuole rimmed by positive material, or a small solid globule. We noted that in several cases thinner sections, more

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carefully stained, dispelled the illusion of DPAS positivity. In some instances, brightly stained stroma insinuated between tumour cells simulated a droplet of secretion.

Table 1. Histochemical reactions of 70 mesotheliomas according to certainty of diagnosis, and of 26 carcinomas

	Mesothelioma			Carcinoma
	Definite	Probable	Possible	
Totals	25	27	18	26
DPAS-positive	0	0	0	12
questionable	0	2	4	0
CI-positive	16	10	2	13
HCl totally removed	12	5	0	0
HCl diminished	4	5	2	0

The importance of preliminary diastase digestion needs no emphasis. Many mesotheliomas have a copious glycogen content.

CI-positivity was accepted only when there was at least a moderate number of staining cytoplasmic vacuoles. The rimming of cell borders, diffuse cytoplasmic staining and minute granules were considered non-specific, and an infrequent globule or vacuole as inconclusive. In addition to its presence in intracytoplasmic vacuoles, the acid mucosubstance was also seen in some cases in coalescent vacuoles, in the lumina of the tubules familiar in many mesotheliomas and in pools in the very myxoid type of intercellular substance. Staining of the connective tissue stroma was not considered to be a diagnostically meaningful reaction.

When the CI preparation was judged positive by the above criteria it was compared with an HCl preparation, and the disappearance of all or a definite quantity of the CI-stained material was noted.

Table 1 discloses that 40% of the cases were CI-positive. In the definite group 64% were positive, in the probable 34% and in the possible approximately 10%. A parallel proportionality seemed to follow in the effect of hyaluronidase. The CI-positive material was totally removed in 3/4 of the definite group, in 1/2 of the probable cases and in neither of the two possible cases.

The fact that 60% of the total series was CI-negative may be attributable either to lack of acid mucosubstance production or to its loss in fixation and/or processing.

By contrast to the mesotheliomas, the CI-positive carcinomas were also DPAS-positive, with one exception. The CI staining was not altered by hyaluronidase in these samples.

DISCUSSION

Our data indicate that while histochemical methods are not the answer to all the problems in the diagnosis of mesothelioma, these methods do play a contributory role.

In the definite cases, where the likelihood of a positive reaction is greatest, it serves as a reassurance and a check on initial classification. Unfortunately, in the merely possible cases where demonstration of the presence of hyaluronic acid would be most helpful a positive reaction is least likely to be present. However, in this area the demonstration of a positive DPAS and of non-hyaluronic acid mucosubstance were helpful in some cases in weighing the decision against a mesothelioma and in favour of a carcinoma. One might mention that some of those mesotheliomas that showed a questionable DPAS also showed acid mucosubstances that were only in part hyaluronic acid, and these cases must be evaluated with caution.

It is in the intermediate group that the method may be most frequently of value. A little over one third of these cases were positive, and in this category the presence of hyaluronic acid may be of particular significance in confirming the evaluation of the case.

In the above study and discussion it must be remembered that we are using the terms mucosubstance and hyaluronic acid purely in their histochemical sense. Spicer *et al.* (1967) have referred to the lack of identity in histochemical and biochemical characterisation of mucopolysaccharides. We must point out that we are not certain of the identity of the DPAS-positive material seen in small amounts in some mesotheliomas, nor of the character of the hyaluronidase-resistant acid mucosubstances.

SUMMARY

Seventy mesotheliomas were studied by the PAS method after diastase digestion, and by the colloidal iron stain, before and after hyaluronidase. Only a few showed a minimal reaction with the former; 40% reacted distinc-

tively with the colloidal iron stain. The diagnostic value of CI staining is limited by the lack of reactivity in 60% of the cases, and particularly in the less typical cases. However, these simple stains are helpful in a sufficient number of instances to warrant their routine use.

ACKNOWLEDGEMENTS

The authors wish to acknowledge gratefully the assistance of Dr Irving J. Selikoff through whom many of the cases were obtained.

Appreciation is expressed to Mr Artie Prado for his excellent technical assistance.

This study was supported by American Cancer Society Grant E-489A and, in part, by USPHS Grant AM-00918 from the National Institute of Arthritis and Metabolic Diseases.

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Diffuse mesothelioma: biochemical stages in the diagnosis, detection and measurement of hyaluronic acid in the pleural fluid

A. BOERSMA,[1] P. DEGAND [1] & R. HAVEZ [1, 2]

The demonstration by Meyer & Chaffee (1940) of hyaluronic acid in the pleural fluid of cases of diffuse mesothelioma provided a biochemical test which seems to be fairly specific for primary pleural tumours.

The "mucin clot" test suggested by Harington *et al.* (1963) has been widely used for the detection of hyaluronic acid, but its sensitivity in detecting that mucopolysaccharide is now in some doubt.

Our investigation of a case of diffuse mesothelioma with viscous pleural fluid led us in 1965 (Tacquet *et al.*) to suggest a method of electrophoretic determination sensitive enough to detect 15 mg of hyaluronic acid per litre of pleural fluid. This study showed that the hyaluronic acid in these effusions is not free; and thus direct electrophoresis of puncture fluid does not allow the determination of this mucopolysaccharide by staining with toluidine blue. The use of a reducing agent (0.075 M cysteine) rapidly lowers the viscosity and releases the hyaluronic acid, which can then be identified by means of its electrophoretic mobility, its affinity for toluidine blue and its sensitivity to hyaluronidase. These three criteria are essential for diagnosis.

From 1965 to 1967 we applied this method of electrophoretic identification of hyaluronic acid in a systematic study of 67 specimens of pleural fluid in cases of various etiology (Havez *et al.*, 1967). In eight cases of histologically confirmed mesothelioma, hyaluronic acid was detected seven times. The fluids were usually of a non-viscous type which does not respond to the precipitation test suggested by Harington. Our method is therefore more sensitive, although diagnosis can be uncertain when only a slight trace of mucopolysaccharide, hardly visible by electrophoresis, is present. It may be emphasised at this point that hyaluronic acid was consistently absent in 23 specimens of pleural fluid from secondary tumours.

Since 1967 we have continued electrophoretic identification of the acid, and we have supplemented this stage of the investigation by measuring the hyaluronic acid in 474 cases of relapsing pleurisy with effusions of a seemingly neoplastic nature but of generally uncertain etiology. Thus, for the present investigation we have studied 65 cases of pleurisy related to mesothelioma which contained hyaluronic acid.

STAGE OF DETECTION

A method for the detection of hyaluronic acid, if it is to be used routinely, must have four essential qualities: simplicity of application, rapidity, adequate sensitivity and specificity.

Our method involves electrophoresis in agar gel (0.9% agar in sodium veronal buffer, pH 8.2, ionic strength 0.1), in which a satisfactory fractionation is obtained after one hour of migration at a potential difference of three volts per cm. The electrophoresis strips are fixed for one hour in a 0.1% solution of Cetavlon, and then dried.

The acidic mucopolysaccharides are stained with a solution of toluidine blue of 40 mg per 100 ml of a 4:1 acetone/distilled water mixture. After 10 minutes of staining the excess dye is removed by washing in a 1% acetic acid solution.

Proteins are stained with amidoblack after fixation

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in a bath of 60% ethanol containing 2% acetic acid. The mobility of serum albumin is a valuable indication for localising hyaluronic acid, since, in pleural fluid reduced by 0.075 M cysteine for two hours at 37°C, the acid migrates slightly ahead of the anode front of that protein.

In addition to electrophoretic mobility a second test is used for the identification of hyaluronic acid, i.e., its sensitivity to testicular hyaluronidase, a property which it shares with 4- and 6-chondroitin sulphates. We use a solution of bovine testicular hyaluronidase (Calbiochem, B-grade) of 1 mg per ml of M sodium acetate in 1.5 M NaCl buffer, pH 5.0. 0.1 ml of this solution is added to 0.9 ml of reduced pleural fluid, and the mixture is incubated for 24 hours at 37°C. Under these conditions the hyaluronic acid is degraded and loses all affinity for toluidine blue.

This method (Havez *et al.*, 1971) has made possible positive diagnosis in 50 cases of pleurisy with hyaluronic acid from 474 cases examined. In 15 cases the presence of hyaluronic acid was suspected but had to be confirmed by chromatographic fractionation. All of these 15 cases had levels of hyaluronic acid between 5 and 10 mg per litre.

Three sources of confusion were encountered when using this test: purulent pleurisy, which gives rise to various types of acidic mucopolysaccharides of cellular origin; previous antimitotic treatment, which reduces the level of hyaluronic acid; and, finally, the collection in error of puncture fluid containing heparin, the presence of which can be demonstrated by electrophoretic study but which sometimes hinders the identification of slight traces of hyaluronic acid.

MEASUREMENT OF HYALURONIC ACID

There is at present no colorimetric method which allows hyaluronic acid to be measured in a complex mixture of glucoproteins; although various methods of purification have been tried, they have either proved too laborious or not specific enough (Scott, 1960).

We have adopted a method whereby 5 ml of reduced pleural fluid are fractionated in a column (25 × 2.3 cm) of Ecteola-cellulose (Cellex E—Biorad) buffered in 0.15 M NaCl. The hyaluronic acid retained on the exchanger is eluted by a solution of 0.5 M NaCl and 0.01 N HCl. The only interference in this fraction is the presence of acid α_1-glucoprotein. Reactivity to orcinol sulphate and to carbazol provide the carbazol:orcinol ratio (0.5 in the case of acid α_1-glucoprotein, and 16 for hyaluronic acid) which confirms the presence of hyaluronic acid and makes it possible to determine its concentration in the fluid under study (Degand *et al.*, 1972).

This method can be applied to the study of tissue biopsies, after hydrolysis of the biopsy specimen by papain and pronase.

The concentrations of hyaluronic acid found varied from 5 to 500 mg per litre. 50% of the fluid specimens examined had concentrations below 50 mg per litre and a total absence of apparent viscosity. The only negative case corresponded to what morbid anatomists call a "pure fibrous" mesothelioma.

We are able to verify that local and general chemotherapy leads rapidly to a fall in the content of this mucopolysaccharide in the effusion fluid or even to its disappearance.

Chemical study of the purified hyaluronic acid confirmed that its structure is highly analogous to that of the hyaluronate-protein isolated from the vitreous body of the eye (Havez *et al.*, 1971).

A single case of epidermoid bronchial cancer in our series was found to have 15 mg of hyaluronic acid per litre of pleural fluid. A case of pleurisy in reaction to bronchiolo-alveolar cancer with hyaluronic acid in the fluid was reported by Izumi & Kurakane in 1971. We have never found hyaluronic acid in pleural fluids or biopsy material from secondary pleural tumours.

The detection of hyaluronic acid in effusion fluid or tissue biopsy material seems to us at the moment to be a decisive stage in the diagnosis of pleural mesothelioma. The ease with which this test can be carried out and its high degree of specificity render it extremely useful in the diagnosis of cases in which the pathological findings are inconclusive.

SUMMARY

(not provided by authors)

The authors describe two screening tests, electrophoretic and chromatographic, for the detection of small amounts of hyaluronic acid in pleural fluids. Samples from 474 cases with repeated pleural effusions were in-

vestigated; 50 definite and 15 possible mesotheliomas were identified by the presence of hyaluronic acid.

In one case of fibrous mesothelioma, no hyaluronic acid was detected in the pleural fluid; whereas hyaluronic acid was found in one case of epidermoid lung cancer and in one case of bronchiolo-alveolar cancer. No hyaluronic acid occurred in any case with a secondary pleural tumour.

The presence of hyaluronic acid in effusions or in tissue biopsies should be one criterion for the diagnosis of pleural mesothelioma, being particularly useful when histology or pathology provide no definite conclusion.

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Diffuse mesotheliomas: diagnostic criteria using exfoliative cytology

E. BLANCHE BUTLER [1] & ANN V. BERRY [2]

The diagnostic value of serous fluid cytology is well recognised, but relatively few cytologists have had experience in the cytodiagnosis of such rare tumours as malignant mesotheliomas.

Standard works on the cytology of effusions (Koss, 1968; Spriggs & Boddington, 1968) contain many references to mesothelial cell morphology and emphasise notable contributions by Klempman (1962), Naylor (1963) and Berge & Gröntoft (1965) on the cytology of malignant mesotheliomas. Koss (1968) also describes findings in 43 cases.

Klempman, reporting on 27 cases, describes differentiated and undifferentiated malignant mesothelioma cells, and states that the tumour can be identified morphologically. Naylor and Berge & Gröntoft are of the same opinion, but their experience is limited to relatively few cases. Koss states surprisingly that in not a single one of his 43 cases was the primary unequivocal diagnosis made on cytological grounds. An interesting case in his series shows morphology which strikingly resembles a synovioma.

Webster (1965), describing the pathology of a large series of mesotheliomatous tumours, advised that consideration be given to the use of exfoliative cytology. His report on cytological examination of 149 effusions lists 35 "positive" cases but gives no morphological description. McCaughey (1965) similarly comments that cytology can be contributory.

Increasing knowledge of the histology of these tumours (Wagner *et al.*, 1960; Webster, 1965; McCaughey, 1965; Churg *et al.*, 1965) has defined the characteristically varied patterns which can arise and which obviously must determine (*a*) whether diagnostic cells will be present at all in an associated effusion; (*b*) the pattern of exfoliation; and (*c*) individual cell morphology.

It is the purpose of this paper to describe the cytology in a further large series of cases as a contribution towards the diagnosis of these rare but ruthless tumours.

MATERIALS AND METHODS

The material studied consists of pleural fluid from 22 cases of malignant mesothelioma seen at the South African Institute for Medical Research since 1962, and 26 specimens of pleural or ascitic fluid from cases of malignant mesothelioma received in Manchester from several centres in the United Kingdom since 1968. The South African material was compared with fluid from 25 cases of mesothelial reaction and 25 cases of metastatic adenocarcinoma. The Manchester material was compared with fluid from 21 cases of mesothelial reaction and 30 cases of metastatic carcinoma; most of the cases were adenocarcinoma, but a few cases of squamous carcinoma were included. In all cases there was histological confirmation of the diagnosis.

The main object of the investigation was to compare and contrast cellular morphology and cell patterns, but in some of the Manchester cases studies were made of mucopolysaccharide and enzyme cytochemistry. The mucopolysaccharide studies were made using consecutive sections in cell blocks. At first these were stained with PAS, PAS + diastase, alcian blue, alcian blue + testicular hyaluronidase and mucicarmine. More recently the methods given

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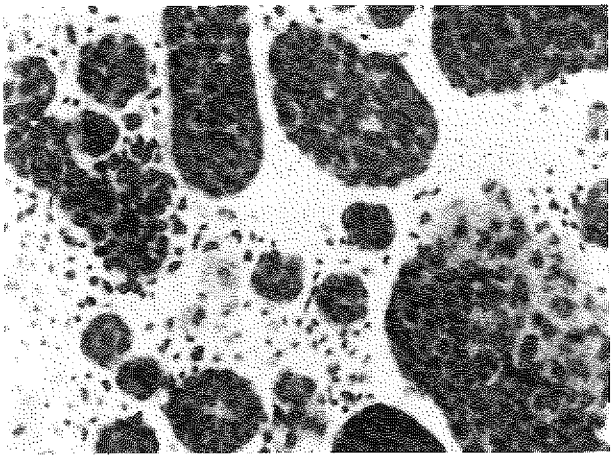

Fig. 1. Malignant mesothelioma; papillary pleomorphic pattern. Tissue fragments are present (some in the form of morulae), also groups and single cells. Large, medium and small cells are present. Papanicoloau's stain; × 100.

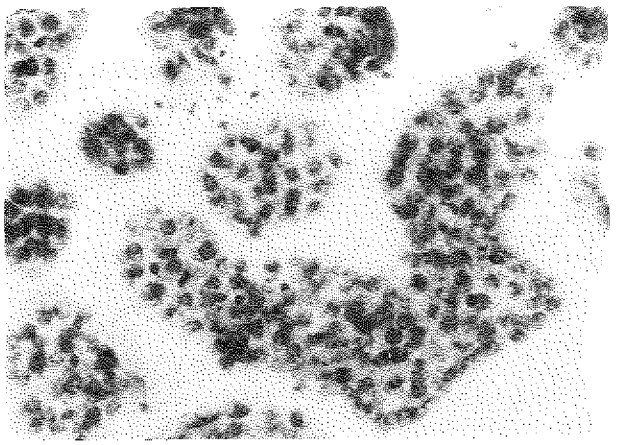

Fig. 2. Malignant mesothelioma; papillary non-pleomorphic pattern. Fragments and morulae consist of small cells of fairly uniform size. Papanicoloau's stain; × 160.

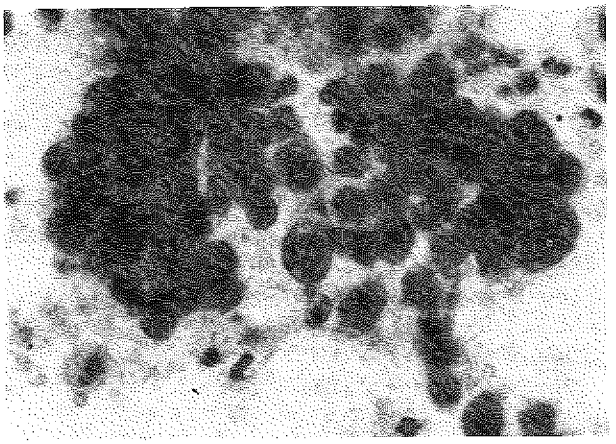

Fig. 3. Malignant mesothelioma; mixed pattern. Cells which vary in size are present in sheets, chains and as single cells. Papanicoloau's stain; × 375.

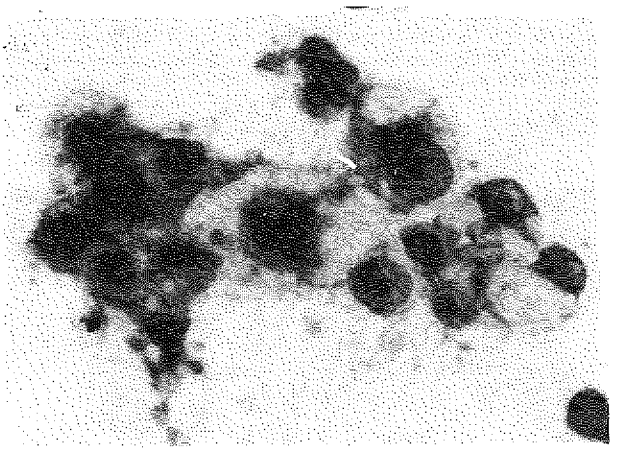

Fig. 4. Adenocarcinoma. A papillary fragment in which the cells contain large irregular vacuoles, nuclei are eccentric and macronucleoli are present. Papanicoloau's stain; × 300.

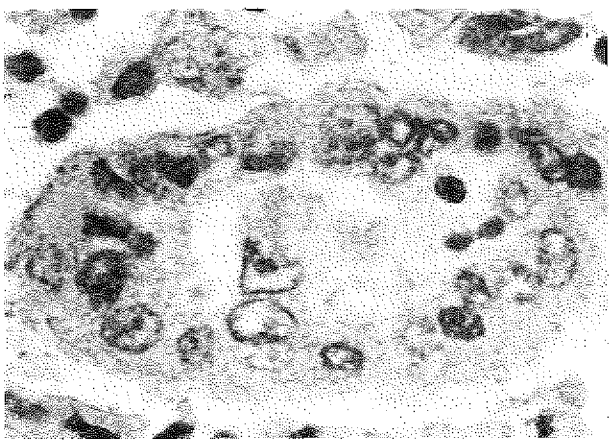

Fig. 5. Malignant mesothelioma; cell block. A regular ring of cells is present. The nuclei are centrally placed, the nuclear chromatin pattern is irregular, and big irregularly shaped nucleoli are present. Nuclei are round or oval and regular in shape. Haematoxylin and eosin; × 800.

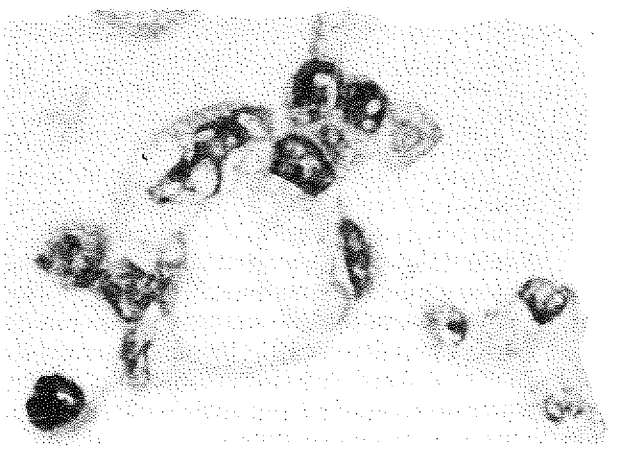

Fig. 6. Adenocarcinoma; cell block. An irregular vacuolated structure is present with large irregular vacuoles. Nuclear chromatin is irregular, and nuclei are present in bizarre forms. Haematoxylin and eosin; × 800.

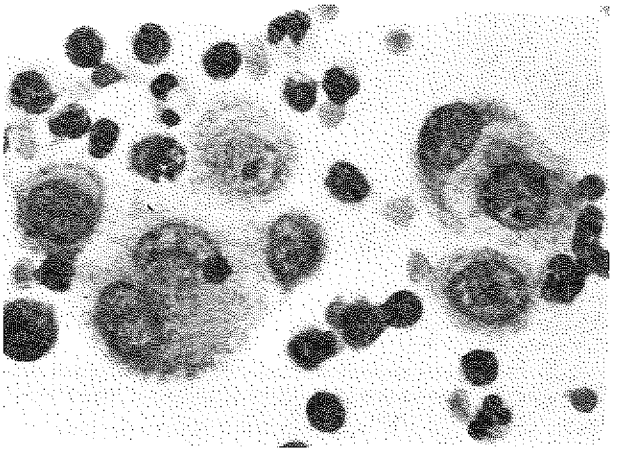

Fig. 7. Benign mesothelial cells. Cells and nuclei are round or oval. The cytoplasm is opaque centrally and fades away at the periphery. Binucleation is present, and single or multiple regular nucleoli are seen. The nuclear chromatin is regular and finely granular. It will be noted that "cannibalism" can be seen in mesothelial reaction. Papanicoloau's stain; × 800.

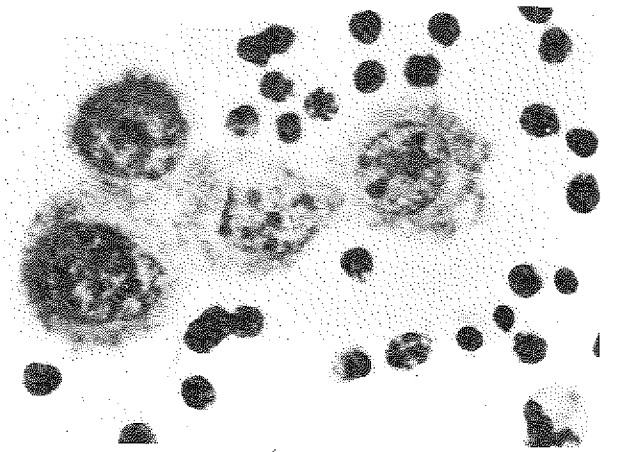

Fig. 8. Malignant mesothelial cells. This group is fairly uniform in size. Nuclei are round or oval and regular. The nuclear chromatin is irregular and coarsely granular. Single or multiple nucleoli are seen which are irregular in shape. The periphery of the cytoplasm has a finely vacuolated lacy appearance. Papanicoloau's stain; × 800.

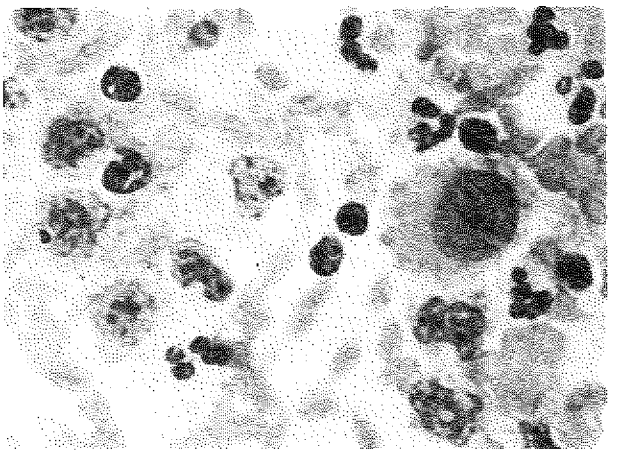

Fig. 9. Atypical macrophage-like cells. These cells have a vacuolated cytoplasm. The nuclei present a variety of bizarre shapes and show folding. The nuclear chromatin pattern is prominent and irregular. A malignant mesothelial cell is seen to the right in the picture. Papanicoloau's stain; × 800.

by Spicer *et al.* (1967) were used; however, these had to be modified as it was not possible to obtain streptococcal hyaluronidase, and treatment of sections with sialidase was also omitted. The methods given by Blonk *et al.* (1967) were used in the study of enzyme cytochemistry, and for this purpose smears were prepared with the cytocentrifuge.

RESULTS

The patients with malignant mesothelioma were Caucasians, with the exception of one African Bantu in the South African group. The proportion of males to females was similar in both centres: the South African cases included 15 men and 7 women, while in Britain there were 21 men and 6 women. The age range was 41–67 in South Africa and 32–79 in Britain; but while 20 of the South African cases were in the 50–55 age group, in Britain only 3 cases fell in this age group and there was no concentration of cases at any age.

Morphology (South African and British cases)

Four cell patterns were observed:

(1) *Papillary pleomorphic* (Fig. 1). Tissue fragments are present, with many rounded into morulae, together with single cells and groups of cells. There is considerable variation in cell size. (South Africa 8, Britain 4.)

(2) *Papillary non-pleomorphic* (Fig. 2). There is profuse exfoliation of uniform small cells in tissue fragments and morulae, with very few single cells. (South Africa 3, Britain 3.)

(3) *A mixed pattern* (Fig. 3). Mesothelial type cells are present in sheets, chains, groups and as

single cells. Exfoliation may be profuse or scanty. (South Africa 8, Britain 11.)

(4) *Single cells.* Single cells are present predominantly, with or without pleomorphism. Exfoliation is usually profuse but may be scanty. (South Africa 3, Britain 8.)

It will be seen that the frequency with which these patterns occurred differed in the two countries. This is not surprising since exfoliation depends upon the relationship of epithelial to mesenchymal elements in the tumour and to the histological pattern of the epithelial growth. These in turn are dependent on host response which could vary with ethnic and geographic factors. It is surprising that in the British cases, in which more than one specimen was received from the same case, 3 out of 6 showed a change of pattern from one specimen to another.

Mesothelial reaction usually presents as single cells or as sheets of cells, but morulae can occur.

Adenocarcinoma usually sheds as papillary pleomorphic fragments, and in most cases the presence of large vacuoles prevents confusion (Fig. 4); but other patterns described occur also. Problems of differential diagnosis arise with some ovarian carcinomas and alveolar adenocarcinoma of lung, and in these cases sections from the cell block are useful. In mesotheliomas regular ring forms are seen (Fig. 5), while in adenocarcinoma irregular acini can be recognised (Fig. 6). Regular rings of cells are also seen in cell blocks from cases of mesothelial reaction, but there are differences in cell morphology (*vide infra*). Difficulties of diagnosis can also arise when a metastatic adenocarcinoma sheds as single cells, and a poorly differentiated squamous cell carcinoma presents the same problem. Here again one is dependent on individual cell morphology.

Individual cell morphology

(1) *Benign mesothelial cells* (Fig. 7). These are round cells containing one or more nuclei, which can be round or oval and which are centrally placed. The outline of the nucleus is regular and the nuclear chromatin is finely granular. Nucleoli are usually present and these also are round or oval and regular in shape. Nucleoli can be large and single, but one of us (A.V.B.) has found that multiple small nucleoli are more common in mesothelial reaction. The cytoplasm is opaque and shades away at the periphery of the cell. In the presence of degeneration large vacuoles can occur, but these have a soft outline and push the nucleus to the periphery of the cell.

(2) *Malignant mesothelial cells* (Fig. 8). The same features of cytoplasmic differentiation as are seen in the benign cells are present, but the nuclei show criteria of malignancy. The nuclear chromatin is more coarsely granular and irregularly arranged. Nucleoli are more likely to be large, single and irregular in shape. The nuclei are still round or oval and centrally placed, but there may be wrinkling of the nuclear membrane with thickening of the chromatin. The shading away of the cytoplasm at the periphery is exaggerated to a fine lacy vacuolation. When large vacuoles appear, due to degeneration, they are similar to those seen in benign mesothelial cells.

(3) *Cells shed from carcinomas* (Fig. 4). These may show distinctive differential characteristics which reflect the structure of cells in the primary tumour. The following features have been found to serve as criteria in cases where the differentiation is less obvious:

(*a*) Vacuolated or foamy cytoplasm. When large single vacuoles are present they have a harder outline, and the nucleus may be moulded around them.

(*b*) Eccentric nuclei.

(*c*) Bizarre nuclei with marked irregularity in shape and variation in size from one cell to another. In the British series this feature was noted in 24 of the 30 cases of carcinoma, but in only 5 of the 26 cases of mesothelioma.

(*d*) Single very prominent macronucleoli are seen in cases of adenocarcinoma.

Mucopolysaccharide and enzyme cytochemistry (British cases only)

There was no distinctive pattern of staining reaction which differentiated mesothelioma from mesothelial reaction or from metastatic carcinoma. Mesotheliomas were less likely than adenocarcinomas to give a positive reaction to PAS after treatment with diastase (in mesothelioma, 6 of 24 cases; in adenocarcinoma, 10 of 25 cases), or to mucicarmine (in mesothelioma, 1 of 16 cases; in adenocarcinoma, 3 of 13 cases). There was no difference in the frequency of a positive reaction between the groups when alcian blue was used at pH 1.0 or at pH 2.5, with or without testicular hyaluronidase. Since it was not possible to obtain streptococcal hyaluronidase the most specific stain available for hyaluronic acid was high-iron diamine alcian blue, which stains blue in the presence of hyaluronic acid and sialomucins (Spicer *et al.*, 1967). This reaction was positive in 4 out of

5 cases of mesothelioma and in 8 out of 9 cases of carcinoma.

The enzyme reactions were almost equally unrewarding. Malignant mesothelial cells and cells from carcinomas were negative for 5-nucleotidase, while 2 out of 4 cases of mesothelial reaction were positive. Malignant mesothelial cells like other malignant cells were more likely to be positive for aminopeptidase than were benign mesothelial cells. Only half of the cases of mesothelioma were positive for isocitric dehydrogenase, while all cases of both carcinoma and mesothelial reaction were positive.

Diagnostic potential of exfoliative cytology (British cases only)

The correlation between histology and cytology is shown below:

Histology		*Cytology*		
		Mesothelioma	*Carcinoma*	*Reaction*
Mesothelioma	27*	25	0	1
Carcinoma	30	2	28	0
Reaction	21	1	1	19

* One specimen was acellular.

Three specimens of fluid sent from cases thought by clinical examination to be mesothelioma were correctly shown to be cases of metastatic carcinoma by exfoliative cytology. Similarly, in three cases in which mesothelioma was not suspected, this diagnosis was made on the basis of exfoliative cytology. It will be noted that the one case of mesothelioma which was missed was thought to be a mesothelial reaction. The two cases of carcinoma wrongly diagnosed as mesothelioma were both anaplastic carcinomas of lung.

Early diagnosis of mesothelioma is important, and an attempt was made to recognise any features in effusions which would draw attention to the disease at an early stage. Six specimens were received from patients with asymptomatic pleural effusion but with a history of contact with asbestos. These fluids were found to contain atypical macrophage-like cells (Fig. 9), similar to cells found in proven cases of mesothelioma. None of these cases has been confirmed histologically, and so they are not included in this survey, but two of them are now considered to show clinical evidence of malignant mesothelioma.

SUMMARY

The diagnosis of malignant mesothelioma using exfoliative cytology is possible. It depends mainly on the recognition of cells which show the cytoplasmic differentiation of mesothelial cells and have nuclei which show the usual criteria of malignancy. Cell patterns are of some assistance, and distinctive features are made clearer by the use of cell blocks. Special stains do not contribute to the diagnosis.

ACKNOWLEDGEMENTS

The investigation was supported in part by the International Agency for Research on Cancer.

It is a pleasure to thank the following who sent specimens of fluid to Manchester: Dr J. R. Edge (Barrow-in-Furness), Prof P. C. Elmes (Belfast), Dr J. C. Hesketh (Portsmouth), Dr J. S. P. Jones (Nottingham), Dr W. G. Owen (Preston), Dr G. Sheers (Plymouth), Dr A. I. Spriggs (Oxford), Dr P. J. Taylor (Liverpool) and Dr H. T. Planteydt (Netherlands).

We should like to acknowledge the technical assistance of Carol Marshall.

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Electron microscopy of normal, hyperplastic and neoplastic mesothelium

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It is generally accepted that mesothelioma is a tumour originating in the mesothelial surface lining. Several histologic forms of mesothelioma are recognised, including epithelial, mixed, biphasic and sarcomatoid (Churg & Selikoff, 1968). Stout & Murray (1942) suggested that both the epithelial and the mesenchymal or fibroblastic tumour cells are derived from a common source, the mesothelial cell, and this suggestion has been reinforced by electron microscopic studies (Luse & Spjut, 1964; Echevarria & Arean, 1968; Stoebner *et al.*, 1970; Mouchel, 1971; Kay & Silverberg, 1971). The present report deals with the ultrastructure of the normal, hyperplastic and neoplastic mesothelium and with the interrelationships among the several forms of the neoplastic mesothelial cells.

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MATERIAL AND METHODS

Normal mesothelial cells were obtained from the pleura of hamsters. Hyperplastic mesothelium was obtained from hamsters with pulmonary asbestosis induced by intra-tracheal injection of chrysotile. In addition, human hyperplastic mesothelium was obtained from the peritoneum surrounding nodules of mesothelioma. These cells were part of the surface mesothelium, as distinct from the tumour, and closely resembled the hyperplastic mesothelial cells which are seen in inflammatory conditions. The neoplastic mesothelium was derived from 10 cases of human mesothelioma (6 pleural and 4 peritoneal). Six of the 10 (4 pleural and 2 peritoneal) had a history of asbestos exposure.

The material was fixed in formaldehyde for light microscopy, and in 2% glutaraldehyde followed by 1% osmic acid, or directly in osmic acid, for electron microscopy. For the latter purpose, tissue was dehydrated with ethanol, embedded in epoxy resin and

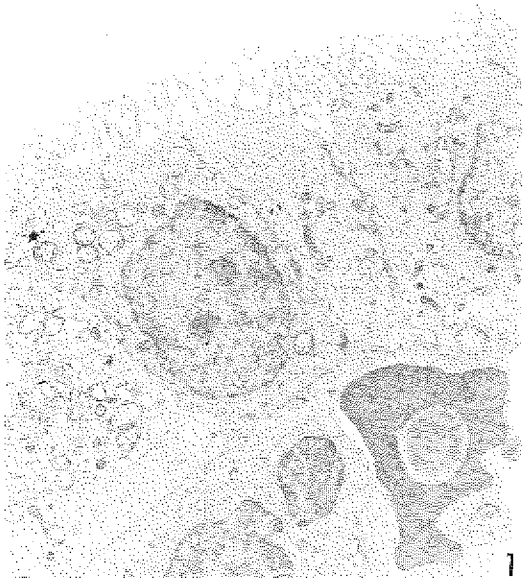
Fig. 1. Peritoneal mesothelioma of a well-differentiated epithelial cell type. Numerous microvilli are seen on the surface of the cells. This architectural arrangement is similar to that of the normal or hyperplastic mesothelium. 5900×

Fig. 2. Peritoneal mesothelioma. A light cell can be seen at the centre of the photograph. It contains multiple nuclei, one of which has a large nucleolus, round mitochondria and a bundle of intracytoplasmic filaments (F). The basement membrane (B) is discontinous beneath the dark cell (D). 4400×

Fig. 3. Part of the mesothelioma cell (taken from the same tissue as shown in Fig. 2). Aggregated glycogen granules are observed. The cell membrane is not invested by the basement membrane. Cross-sections of collagen fibrils (C) are seen around the cell.

Fig. 4. Pleural mesothelioma. The tumour cells are low, however they still form a tubular lumen (L) and possess a small number of microvilli (M), junctional structures (J) and a basement membrane (B). A tumour cell (X) has lost some of its epithelial features, although the general appearance of its organelles is similar to that of an epithelial cell. 6000×

Fig. 5. A high-power view of the junctional area shown in Fig. 4. A desmosomal structure is seen. Tonofilaments spread into the cytoplasm from the desmosome. 48,000×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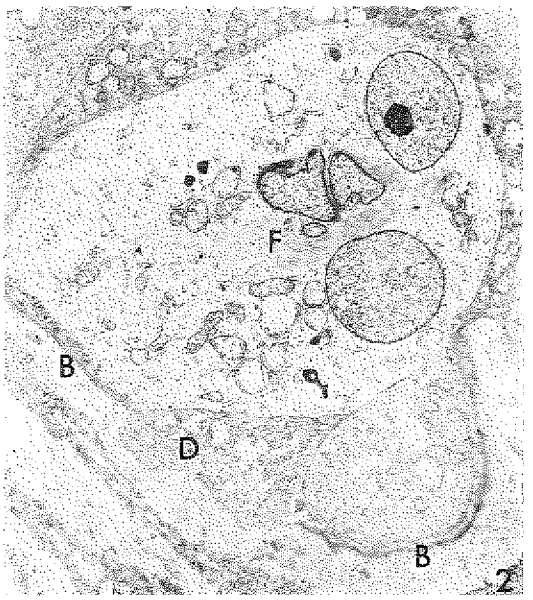

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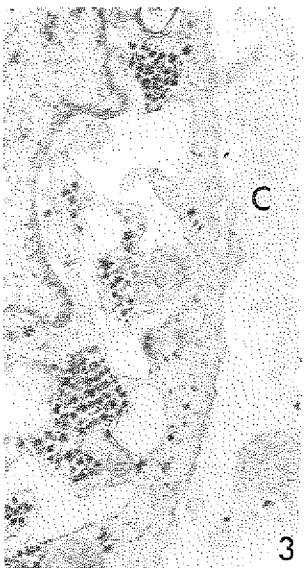

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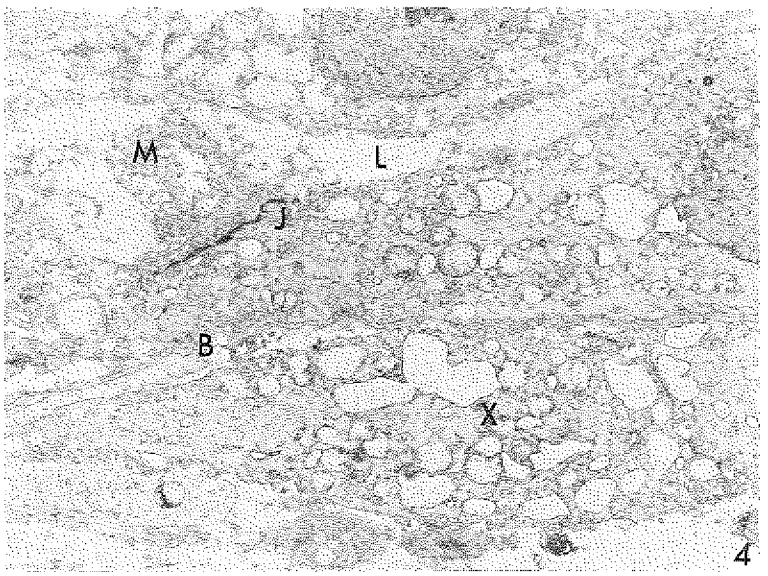

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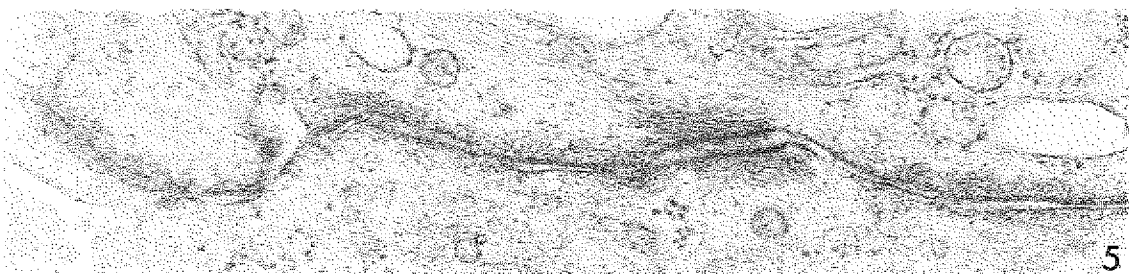
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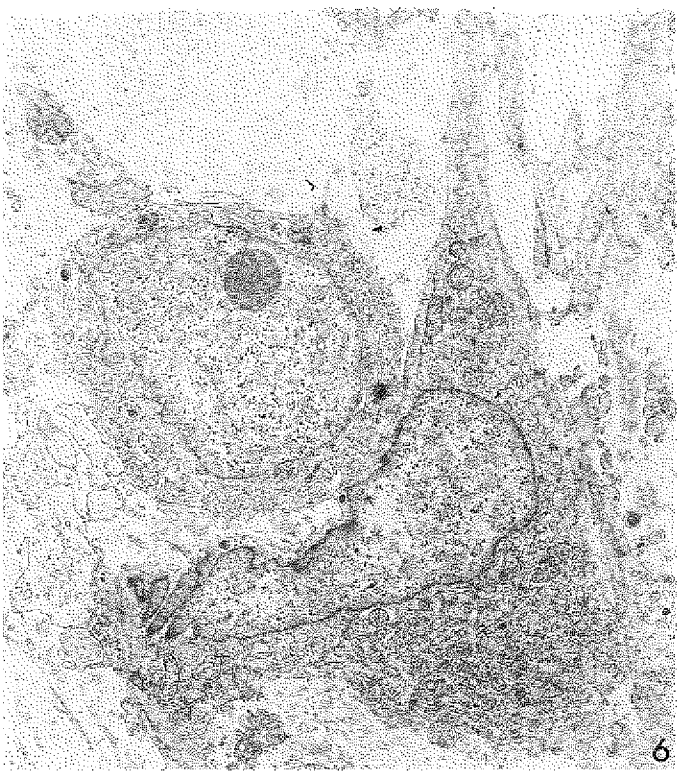

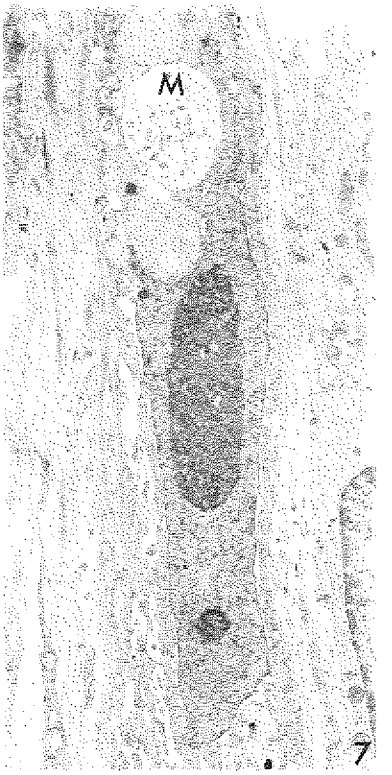
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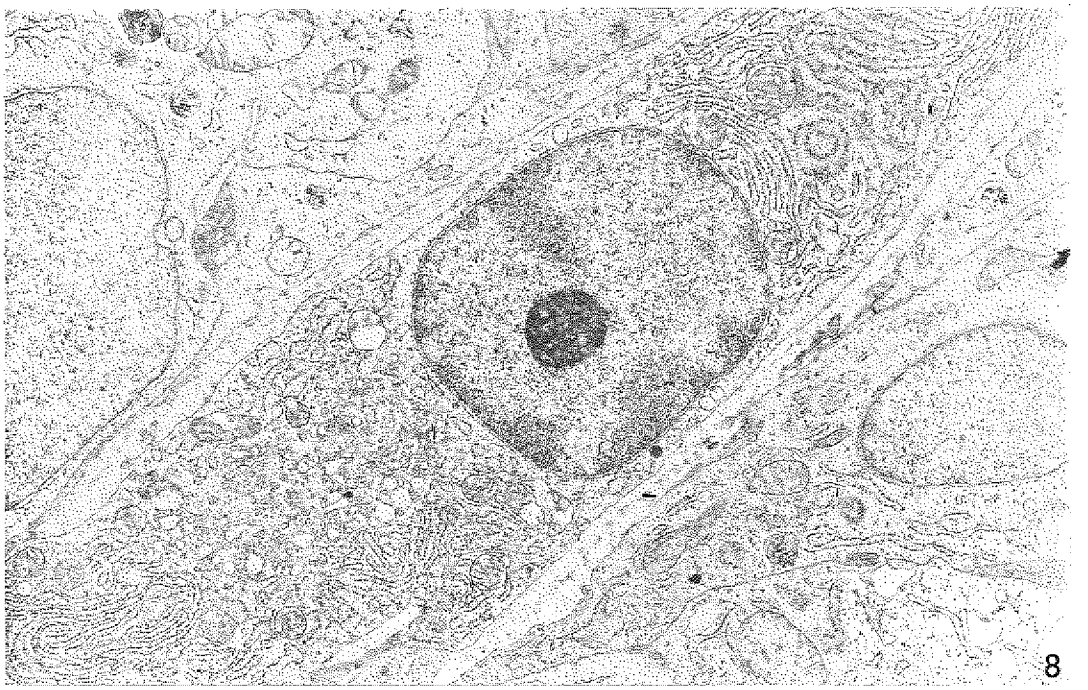

studied in RCA 3G and Hitachi 11-ES electron microscopes.

RESULTS

The normal mesothelium of hamster consists of flat cells equipped with short microvilli, many pinocytotic vesicles and ovoid nuclei. Tight junctions connect the adjacent cells. There are no bundles of intracytoplasmic filaments or glycogen granules. The cells rest on a thin basement membrane which separates them from the submesothelial space. The latter contains collagen fibrils, thin microfibrils, elastins and connective tissue cells which are similar in appearance to the alveolar septal cells of the underlying lung. Often the submesothelial tissue appears to be continuous with the alveolar septa.

The hyperplastic pleural cells of the hamster have voluminous cytoplasm containing well-developed cell organelles, such as rough-surfaced endoplasmic reticulum, Golgi complex, pinocytotic vesicles and a few cytosomes. The nuclei are large. In addition to the tight junctions, desmosomes are frequently seen. Microvilli are more numerous and are longer than those in the normal mesothelial cell. The intercellular spaces are often widened and the basal cell membrane is separated from the basement membrane. The latter is often discontinuous.

Human hyperplastic mesothelium is quite similar in its ultrastructure to that of the hamster. Such cells may be difficult to distinguish from the cells of a well-differentiated mesothelioma of the epithelial type.

On the basis of examination by light microscopy the ten mesothelioma cases could be divided into three groups: (1) *the epithelial*, consisting of 5 pleural and 3 peritoneal tumours and composed mainly of epithelium-like cells; (2) *the mixed*, consisting of typical and atypical epithelial cells (1 peritoneal tumour); and (3) *the biphasic*, containing two apparently different elements, the epithelial and the mesenchymal (1 pleural tumour). There were no purely mesenchymal or sarcomatous tumours in this series. The epithelial tumours exhibited a variety of architectural patterns: adenomatous, tubular, papillary and cord-like. In a number of cases many individual epithelial cells were scattered in the connective tissue stroma of the tumour. In addition, about half of the cases of the epithelial type also had a small number of non-epithelial, mesenchymal tumour cells, such as are seen in biphasic mesotheliomas. Echevarria & Arean (1968) find that "mixed" or "biphasic" patterns can quite commonly be seen in the electron microscope.

The neoplastic mesothelial cells of the epithelial type vary strikingly in size and shape. They are usually equipped with microvilli, junctional structures, intracellular filaments, large ovoid mitochondria and well-developed rough-surfaced endoplasmic reticulum (Figs. 1 to 5). In some cases the cells also contain large amounts of glycogen (Fig. 3). The Golgi complex and lysosomal granules are poorly developed and secretory granules are rare. Osmophilic lamellar bodies are found occasionally, but they are small and few in number. The amount of ribosomes varies considerably, causing cells to have "dark" or "light" cytoplasm (Fig. 2). Both intra- and intercellular caveolae are common; they are equipped with well-developed microvilli. Basement membranes may be partially or completely absent (Fig. 3). In one case of peritoneal mesothelioma, myxovirus-like particles were found in the cell cytoplasm. Nuclei of the tumour frequently include well-developed nucleoli.

The atypical epithelial cells seen in the "mixed" forms of mesothelioma lack some of the epithelial features, such as polarity, junctional complexes, microvilli and basement membranes. They are often elongated, resembling fibroblasts, and probably represent intermediate forms between the epithelial and the mesenchymal (non-epithelial) tumour cells (Figs. 4, 6, 7). The latter constitute one phase of the "biphasic" mesothelioma and are frequently indistinguishable from fibroblasts (Fig. 8).

Fig. 6. Pleural mesothelioma. Two tumour cells lying free in loose connective tissue. They are the intermediate form between the epithelial and non-epithelial (mesenchymal) cell types. 5900×

Fig. 7. Peritoneal mesothelioma. Another intermediate cell which has an intracellular caveola containing microvilli (M) and an extremely elongated cytoplasm. 4400×

Fig. 8. Pleural mesothelioma. A large non-epithelial (mesenchymal) cell is seen at the centre of the photograph. It is similar to the fibroblast in its structure and is surrounded by negatively stained collagen fibres. 5600×

DISCUSSION

The ultrastructure of hamster mesothelium does not differ from that of other species, such as rat (Odor, 1954), mouse (Felix & Dalton, 1956; Rhodin, 1963) and man (Salazar *et al.*, 1972), except that basement membranes are sometimes poorly defined. It is noteworthy that in the pleura the submesothelial connective tissue is almost identical in structure with that of the pulmonary alveolar septa, and that the two structures are often continuous. This suggests that both may be similarly affected by pathological processes.

The ultrastructure of the hyperplastic mesothelium in human pleura has been studied by Stoebner *et al.* (1970), who found abundant microvilli, glycogen granules and intracellular filaments, and noted morphologic similarity between the hyperplastic and the neoplastic mesothelial cells. Our material suggests that this similarity obtains only for the well-differentiated tumour cells of the epithelial type, and that glycogen is often scanty or absent.

The literature contains reports of 13 cases of mesothelioma studied by electron microscopy (Luse & Spjut, 1964—1 case; Echevarria & Arean, 1968—2 cases; Marcus & Lynn, 1970—2 cases; Stoebner *et al.*, 1970—3 cases; Kay & Silverberg, 1971—1 case; Mouchel, 1971—2 cases; and Salazar *et al.*, 1972—3 cases). There is, however, no unanimity on the characteristic features of the neoplastic mesothelial cell, perhaps because it varies greatly in appearance. The findings in our 10 cases are in essential agreement with the previously reported features of the mesothelioma cell of the epithelial type, including the presence of microvilli, junctional structures, dark cells (rich in rough-surfaced endoplasmic reticulum) and light cells (containing bundles of intracellular filaments), glycogen granules, well-developed nucleoli and continuous or discontinuous basement membranes. In our series, however, electron dense secretory granules were rarely seen and osmophilic lamellar bodies were not always present. One may also add, in agreement with others (Marcus & Lynn, 1970; Mackey *et al.*, 1971; Salazar *et al.*, 1972), that epithelial mesothelioma cells closely resemble those of adenomatoid tumours of the male and female genital tracts.

The non-epithelial or mesenchymal cell seen in the so-called "mixed" and especially in the "biphasic" types of mesothelioma is also believed to originate in the surface mesothelium. This view is favoured by most electron microscopists (Luse & Spjut, 1964; Echevarria & Arean, 1968; Kay & Silverberg, 1971), and is based on the old tissue culture studies of Maximow (1927) and Stout & Murray (1942). These cells are capable of laying down fibrous tissue.

The transformation of the neoplastic mesothelial cell of the epithelial type into the mesenchymal type has been followed by Stoebner *et al.* (1970) and by Mouchel (1971), who observed dissociation of individual cells along the periphery of tumour nodules. The present study also confirms the existence of transitional forms, from typical epithelial to atypical epithelial, and finally to non-epithelial or mesenchymal mesothelioma cells.

SUMMARY

The ultrastructure of normal (hamster), hyperplastic (hamster and human) and neoplastic (human) mesothelium was examined by electron microscopy. The neoplastic cells, as seen in 10 cases of mesothelioma (6 pleural and 4 peritoneal), exhibit striking structural variability. Three major cell types, the epithelial, the atypical epithelial and the mesenchymal or fibroblastic are distinguished; and a variety of intermediate forms among these three cell types were recognised. It is concluded that "mixed" and "biphasic" patterns of mesothelioma are derived from a single type of tumour cell which originates in the mesothelium.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance of Dr Irving J. Selikoff, through whom most of the mesothelioma cases were obtained. Appreciation is expressed to Dr Tetsuro Ono and Mr Norman Katz for their excellent technical assistance.

This study was supported by USPHS Grants AM-00918 and UI-00496.

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Discussion summary

H. T. PLANTEYDT [1]

Although progress in the development of pathological methods for the assessment of the biological effects of asbestos seems to have been rather slow, a considerable amount of information has been collected and the value of certain techniques has been assessed.

There was wide ranging discussion on the quantitation of asbestos bodies. There was a need for standardisation of criteria since observers still showed considerable variation even when the same technique was used, and a single observer often gave very different counts when using different techniques on the same material. A simple semi-quantitative approach was proposed, using routine histological sections of lung. The results would be recorded in the following manner:

Class 0: no asbestos bodies;
Class 1: very sporadic appearance, difficult to find one body in a slide;
Class 2: few bodies, easy to find, but not in every field;
Class 3: many bodies, easy to find at least one in every high power field;
Class 4: very many bodies, usually many bodies in each field.

Although this technique would not give the actual number of bodies, it might yield reproducible results. Possibly these could be compared with the categories used by Pooley[2], and with the various groupings for occupational exposure. Standard slides could be very useful in making observations comparable.

The specificity of asbestos bodies was considered. It was generally felt that although other types of fibre have been found in "ferruginous bodies", the pathologist's diagnosis of asbestos bodies had been reasonably reliable as in the majority of cases investigated they had been shown to contain asbestos fibres. However, in most publications the description of asbestos bodies was inadequate, and no information was available to define a pseudo-asbestos body.

Electron microscopy was felt to be a more accurate method of assessing the number as well as the character of fibres in the lung. However, there was no agreement as to whether the proportion of fibres seen with the light microscope and that seen under the electron microscope was constant enough to be reliable. It should be borne in mind that chrysotile fibres can only be reliably detected in the lung by electron microscopy. Counting and measuring of asbestos fibres using the electron microscope must therefore be the method of choice if an accurate count is required; then the use of two different techniques of preparation of specimens is recommended: paraffin slides and KOH-digested tissue.

The difference in magnetic properties of the various types of asbestos fibres may form the basis of new methods for identifying and even quantifying these fibres.

The morbid anatomical and histological diagnostic criteria for diffuse mesothelioma have been more clearly established. Although there was considerable observer variation in assessing detailed histological features, it was agreed by the pathologists that a reliable diagnosis can be made in 70–90% of mesotheliomas, based on:

(*a*) recognition of the capacity of the malignant mesothelial cell to form widely different forms of neoplastic tissue;

(*b*) a familiarity with the more specific structural patterns of the tumour; and

(*c*) a good knowledge of general tumour pathology.

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[2] See p. 50 of this publication.

The gross appearance of the tumour can be of great value in making a diagnosis, particularly in the pleural cavity where diffuse mesotheliomas most frequently occur. It is extremely rare for secondary tumours to reproduce the appearance of late-stage mesothelioma, in which a sheet-like layer of growth largely, or completely, replaces the visceral and parietal pleura and encases the lung. Spread of the tumour to involve the regional lymph glands and the underlying lung and liver is well known. More recently it has been agreed that discrete metastases may occur; these have been most commonly observed in the lungs and liver, but are occasionally more widespread.

In a study of the Canadian material, Professor Magner demonstrated that the mixed mesothelial pattern was most common in cases where there had been occupational exposure to asbestos. It was suggested that these findings should be checked in other countries. A number of pathologists stated that the vast majority of these tumours would show a mixed pattern if sufficient sections were taken.

It was hoped that the electron microscope investigations would be extended to ascertain if this could be used as a diagnostic tool. However, it was pointed out that in the majority of cases specimens are not received in suitable condition for this method.

There was a need for standardisation of the criteria for the diagnosis of mesothelioma by exfoliative cytology, because if this method could be well established diagnosis could be made on patients without the necessity of taking a biopsy. The elaboration of this technique by cytochemical and enzyme studies was mentioned, but the work on both these aspects is still in a preliminary stage.

The information that more elaborate biochemical studies are available for the demonstration of hyaluronic acid in pleural fluid was welcomed and it was hoped that these methods would be more widely used, and that there would be collaboration between the biochemists and the pathologists carrying out histochemical studies for the presence of this material.

ASSESSMENTS OF METHODS USED IN THE STUDIES OF THE BIOLOGICAL EFFECTS OF ASBESTOS. 3. EXPERIMENTAL PATHOLOGY

Chairman — J. Wyatt

Rapporteur — J. S. Harington

Investigations using animals

J. C. WAGNER [1] & G. BERRY [1]

In 1964, the UICC Working Party considered the results of the experimental studies which had been reviewed, or which had been presented, at the preceding New York Academy of Sciences Symposium (UICC, 1965). "It was agreed that various types of asbestos induced in many species of animals lesions similar to those seen in human cases of asbestosis, mesothelioma and possibly carcinoma of the lung, but the need for more precisely planned experiments was emphasised.

"The desirability of using healthy animals of known response to asbestos under quantitative conditions of exposure by inhalation, feeding and parenteral injections at several sites was agreed." To carry out these recommendations there were certain basic requirements which had to be met.

TYPE OF ANIMAL

The type of animal to be used was widely discussed, and the majority of opinion was in favour of the caesarean-derived (C/D) rat strains which were free of murine bronchitis (SPF) and kept under barrier controlled conditions. This policy has been effectively pursued in Britain and is being used in four research units undertaking asbestos experiments. A similar unit has been established in South Africa, and it is hoped that other units will follow. The animal being used in the British and South African units is the C/D Wistar rat of the Alderley Park (ICI) strain. However, the need for additional strains is realised, and these are being bred. An interesting development has been the use of large primates (baboons), which are being treated at the South African Medical Research Council's Unit in Johannesburg. Smith *et al.* (1965) used hamsters, since their lungs are free of disease, and we recommended C/D rats for the same reason.

[1] MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, UK.

LENGTH OF EXPERIMENT

The need for long survival after the initial treatment was emphasised by Smith at the NYAS Conference, and was indicated by our preliminary results (Wagner, 1965). We both emphasised that the animals should be allowed to survive their full life-span.

In six intra-pleural inoculation experiments at the MRC Pneumoconiosis Unit, which gave a total of 803 mesotheliomas, only 20% of tumours occurred within 18 months after inoculation. The incidence was greatest after 2 years, by which time two-thirds of the total number of tumours had been seen; by 30 months after inoculation, 95% of the mesotheliomas had occurred. Hence, it is clear that most of the information would have been lost if the experiments had been terminated after 18 months; even termination after 2 years would have been premature. The experiments could have been terminated after 30 months with no serious loss of information; but at this stage most of the rats were dead, and the experiments were so near to their natural conclusion that such a termination could be considered only as a tidying-up operation rather than as a basic experimental strategy.

If the cost of carrying out an experiment is considered both as overheads per animal and running costs of keeping each animal then it can be shown, provided only that the age-specific death rate of animals dying with a mesothelioma increases with time after injection (a condition which is fulfilled in our experiments), that the best return per unit cost is yielded by allowing all the animals to live out their lives. However, in a number of reports that have

been presented since 1964, the animals have either died of inter-current disease or have been deliberately killed before they have reached the period of maximum tumour production.

DUST TO BE TESTED

On the basis of the UICC recommendations relating to chemistry and physics, the standard reference samples of the more important asbestos deposits were prepared and have been fully characterised (Timbrell [1]). These samples have been criticised in personal discussions on a number of grounds; the queries regarding their physical properties are dealt with in Dr Timbrell's paper. The main biological objections were that:

(*a*) the samples were not representative of the dusts to which workers were exposed;

(*b*) it was not possible to use the whole sample for intra-pleural inoculation since the material, particularly the chrysotiles, blocked the needle; and

(*c*) the material is unsuitable for intra-tracheal inoculation since it blocks the trachea and major bronchi.

It must be emphasised that these samples were prepared to provide a base-line for subsequent investigations, and workers were encouraged to standardise their results by using the appropriate reference sample as a control. They were meant to provide a high percentage of respirable dust and were designed mainly for inhalation, parenteral inoculation and feeding experiments. The criticisms regarding intra-pleural inoculations have been overcome by personal demonstration at a number of laboratories. It was not intended that these samples should be used for intra-tracheal inoculation studies.

METHODS OF EXPOSURE

Intra-pleural inoculation

This is the simplest means of obtaining pleural mesotheliomas (Wagner & Berry, 1969), but it is unphysiological, since in direct application to the intra-pleural space both the defence mechanisms of the pulmonary tissues and the aerodynamics of the respiratory tree have been by-passed.

[1] See p. 295 of this publication.

Intra-pleural implantation

This modification has been developed by Stanton *et al.* (1972) and opening of the thoracic cavity allows for a more specific application to the visceral pleura. Considerable surgical skill is required if a significant number of animals are to survive for a long period.

Intra-tracheal inoculation

This method has been most frequently used in testing the toxicity of dusts *in vivo*. It is not a satisfactory means of testing the effects of asbestos dusts on the lung parenchyma. In addition, finely divided chrysotile fibres form a gel in aqueous solutions, so that if chrysotile is introduced in this manner it will obstruct the upper respiratory tract and suffocate the animal.

Inhalation

The most realistic way of exposing animals to various types of dusts, as it most closely parallels the human situation, is by inhalation. For the results to be of value, the dust cloud must be uniform, accurately monitored, and there must be a method for assessing the amount of dust retained by the animals. Thus, reliable inhalation experiments require the collaboration of physicists, chemists and experimental pathologists.

The production and testing of dust dispensers, chambers and methods of calibration have been described (Timbrell *et al.*, 1968; Timbrell *et al.*, 1970).

Intra-peritoneal inoculation

The intra-peritoneal inoculation of various types of asbestos dust has been undertaken in rats and mice in an attempt to produce peritoneal mesotheliomas (Gross *et al.*, 1970; Reeves *et al.*, 1971).

Subcutaneous inoculation

This method was used by Roe *et al.* (1967) for investigating the co-carcinogenic effect of various types of asbestos. The difficulty in this procedure is the frequency with which the fibre escapes by ulceration through the over-lying epidermis. In their initial studies they found that a considerable amount of the inoculated fibre tended to track through the tissues to mesothelial surfaces. This was not confirmed by later, more sophisticated, studies (Kanazawa *et al.*, 1970; Morgan & Holmes, 1970; Morgan *et al.*, 1971).

Feeding experiments

Bonser & Swinburne [1] have mixed asbestos with food; and recently, Morgan & Holmes [1] have been studying the passage of irradiated fibre through the gut of rats. In a collaborative experiment, animals are being fed with known amounts of crocidolite fibre during a six-month period, after which they are being allowed to survive their natural life span.

The use of irradiated fibre (Morgan & Holmes, 1970) has also been of value in calibrating dosage in the intra-pleural, intra-tracheal, inhalation and subcutaneous studies.

DISCUSSION

One of the disappointments of the experimental studies of the biological effects of asbestos over the last six years has been the modification by a number of people of the UICC reference samples, either by further grinding of the material or by separating it into different fractions by elutriation, but still regarding these as equivalent to the standard samples. If these modified materials were compared for biological effect with the original standard samples, valuable information could be obtained; but if they are used alone the results can only be considered as those of uncontrolled studies. It should be emphasised that if samples are ground or re-milled, the danger of metal contamination is an additional complication; this has been clearly illustrated by Gross *et al.* (1967).

There are numerous laboratories which have not used the standard reference samples of dusts for their basic investigations; this does not invalidate their results, but it makes comparison with other investigations difficult. Therefore, it is strongly recommended that the appropriate UICC reference sample or samples be used as control dust in further experimental studies.

[1] Personal communication.

SUMMARY

The various methods that have been used in attempts to produce tumours in experimental animals are reviewed. The necessity of keeping the animals for a sufficiently long period after exposure is emphasised. The advantages of exposing the animals to the UICC standard reference samples of asbestos dust are indicated and their use in further studies recommended.

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Experimental methods—cell and tissue culture: effects of asbestos particles on macrophages, mesothelial cells and fibroblasts

A. C. ALLISON [1]

Four cell types are potential targets for asbestos *in vivo*: (1) *macrophages*, especially pulmonary alveolar macrophages; (2) *mesothelial cells*, which undergo malignant transformation; (3) *fibroblasts*, which participate in the fibrogenic reaction; and (4) *pulmonary alveolar epithelial cells*, which also undergo malignant transformation. The biological effects of different types of asbestos depend partly on the size and shape of the particles, which determine what proportion are deeply inhaled into the bronchial tree, penetrate to the mesothelium, and can be ingested by cells, and partly on the chemical composition of the particles, in particular of their surfaces. Because there are so many different types of asbestos, and each one can be obtained in a range of particle sizes, a comprehensive study of their activities upon various cell types would be a large undertaking. My colleagues and I have made fairly extensive studies of the effects of different types of silica on peritoneal macrophages; and I have extended these studies to investigate some effects of asbestos on macrophages, mesothelial cells and fibroblasts. The experiments have led to preliminary conclusions (Allison, 1971), which agree quite well with those of Bey & Harington (1971) but differ somewhat from those of Parazzi *et al.* (1968).

CULTURES

Unstimulated mouse peritoneal macrophages were cultured as previously described (Allison *et al.*, 1966). Each culture contained 10^6 adherent cells, mostly macrophages, on a coverslip 22 × 7 mm, in 1 ml of medium. Cells were incubated overnight, and the medium was changed before the cells were exposed to dusts.

Organ cultures of peritoneum from weanling mice were prepared; in mice of this age the peritoneum is thin and has little fat. The peritoneum supporting the small intestine was excised, cut into small pieces about 3 mm square, laid on a nylon-mesh grid and placed on a support in a Nunc plastic Petri dish. The peritoneum tended to curl up, but if the ends were tucked under the nylon mesh, this difficulty could be overcome. Medium (199 + 10% foetal calf serum) was poured into the dish until the level of the peritoneum was reached, and the samples were equilibrated with a mixture of 90% N_2, 5% O_2 and 5% CO_2. (Mesothelial cells appear to be more sensitive to oxygen than most cells; in the course of a few days' culture at atmospheric oxygen concentrations they developed brown granules and their condition deteriorated.) The cultures were examined at daily intervals by phase-contrast microscopy. Asbestos fibres were allowed to sediment onto the peritoneal cultures from the medium, which was then removed.

The viability of the cells after exposure to dusts was measured by the loss of their capacity to split fluorescein diacetate (Rotman & Papermaster, 1966). Histochemical studies for acid phosphatase, by the Gomori method as previously described (Allison *et al.*, 1966), and for β-glucuronidase, by the method of Hayashi *et al.* (1964), were performed on all cells. Release of the cytoplasmic enzyme lactate dehydrogenase (LDH) and the lysosomal enzyme β-glucuronidase (β-gluc) into the medium was also measured.

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SIZE OF ASBESTOS PARTICLES TAKEN UP BY PHAGOCYTOSIS

The first question to be considered was the limit of size of particles that could be ingested by phagocytosis. Fractions of chrysotile and crocidolite containing high and low percentages of short fibres were incubated with the cells in medium 199 containing 10% foetal calf serum for 3 hours at 37°C. The cells were examined by time-lapse phase-contrast cinephotomicrography, interference microscopy, and, after fixation in glutaraldehyde (2% in 0.05 M sodium phosphate, pH 7.0, for 2 hours at room temperature), they were examined by stereoscan electron microscopy. The two last-mentioned techniques were useful in assessing whether the particles were inside the cells or outside, which is sometimes difficult to establish by conventional microscopy.

Independently of the asbestos type, short fibres (< 5 μm) were readily and completely taken up by phagocytosis, whereas long fibres (> 25 μm) were never completely taken up. The cells were closely attached to or enveloped the ends of the latter, but part of these long asbestos fibres remained outside of the cells. The reflection of the plasma membrane over the particle was demonstrable in stereoscan electron micrographs. With long fibres, two or more cells could be seen attached to a single fibre, sometimes with apparent continuity of cytoplasm, and the presence in the culture of multinucleate cells containing long fibres suggests that the process may lead to cell fusion. Particles of intermediate size (5–25 μm) were sometimes completely ingested and sometimes not. Silica differs from asbestos in that all the inhaled silica particles (which are nearly isometric) reaching the pulmonary alveoli can be ingested by macrophages, whereas some inhaled asbestos particles are probably not completely ingested.

INTERACTION WITH PLASMA MEMBRANES

It is well established that when silica particles suspended in an appropriate buffer, such as barbiturate (but not Tris or phosphate), and in the absence of serum or other body fluids containing macromolecules, are added to red blood cells or macrophages they rapidly lyse the cells by interaction with the plasma membrane. A similar interaction takes place with phospholipid-cholesterol bi-layers in the form of liposomes. The most likely explanation of the underlying mechanism of this phenomenon is that the rigidly placed phenolic hydroxyl groups on the surface of the silica particle are able to form multiple hydrogen bonds with the phosphate ester groups of membrane phospholipids, and probably also with secondary amide groups of proteins, thereby distorting the membrane and increasing its permeability. Compounds such as polyvinylpyridine-N-oxide, which can readily form hydrogen bonds with silicic acid and so prevent its interaction with membranes, protect cells from lysis by silica (Allison, 1971).

Certain types of asbestos fibres are also haemolytic and are rapidly cytotoxic to macrophages cultured in media lacking serum (Harington *et al.*, 1971). Precise comparison of the effects of the different types of asbestos is not possible because size, shape and surface area cannot be matched; but it is possible nevertheless to make a clear correlation as to haemolytic potency, macrophage cytolytic potency and concentration of magnesium for the various fibres. Thus, chrysotile and anthophyllite are highly active in both systems, whereas amosite and crocidolite are not (I have no explanation for the report of Parazzi *et al.* [1968] that crocidolite is more cytotoxic than chrysotile, which is in conflict with other reports). Further evidence that magnesium on the surface of asbestos fibres plays an important role in their interaction with membranes comes from a study of the effects of compounds that combine more or less selectively with different groups on the surface of the particles or cells (Table 1). As shown by Macnab & Harington (1967), polyvinylpyridine-N-oxide (PVPNO), which protects against silica haemolysis, has very little effect against chrysotile haemolysis; whereas ethylenediaminetetraacetate (EDTA), which chelates many metal ions and especially calcium and magnesium, strongly inhibits haemolysis. Since this effect might have been exerted on Ca^{++} ions known to be associated with the cell coat, I examined the effects of ethylenebis(oxyethylenenitrilo)tetraacetate (EGTA), which has a much higher affinity for Ca^{++} than for other ions, on the reaction; little effect was observed. In contrast, the selective Mg^{++} chelating agent, sodium-1-azo-2-hydroxy-3-(2,4-dimethylcarboxanalido)-naphthalene-1′-(2-hydroxybenzene-5-sulphonate) ("Magon"), markedly depressed chrysotile haemolysis. These results strongly suggest that magnesium hydroxide groups, known to exist in a brucite-type array on the surface of the particle, are mainly responsible for their interaction with membranes.

Table 1. Effects of various agents on haemolysis by chrysotile and quartz[a]

Chrysotile	67 ± 4.2
" + PVPNO	63 ± 5.1
" + EDTA	17 ± 3.2
" + EGTA	61 ± 4.7
" + Magon	22 ± 4.3
Quartz	78 ± 5.7
" + PVPNO	13 ± 3.2
" + EDTA	74 ± 6.1

[a] 10 mg UICC chrysotile and 10 mg quartz, barbiturate-buffered saline, pH 7.0, in 1 ml sheep erythrocytes, 10 min at 37°C.

It is likely that the major interaction of asbestos is with membrane sialoglycoproteins. Harington *et al.* (1971) have shown that enzymic removal of sialic acid groups decreases the susceptibility of sheep erythrocytes to crocidolite haemolysis. I have found that crocidolite haemolysis is markedly antagonised by the presence of isotonic polyvinylpyrrolidine in the medium. This does not appear to be an effect on the particles themselves, as it does not occur when they are pre-treated and washed, but is probably an osmotic effect. It is now known that membrane siaologlycoproteins do not occupy a fixed position, but that they have lateral mobility in the plane of the membrane. The most likely explanation of asbestos haemolysis appears to be that membrane sialoglycoproteins interact with asbestos fibres and form a cluster beneath the fibres. As discussed elsewhere (Allison, 1972), such protein clusters could create channels through which small molecules, but not proteins, can readily move. The membrane becomes more permeable to ions and water, and the osmotic effect exerted by the haemoglobin trapped within the cell causes it to swell and burst.

This is, of course, a model for interaction of asbestos fibres with biological membranes, either the plasma membrane of target cells or the membrane surrounding the secondary lysosomes. As previously shown (Allison, 1971), incubation of asbestos fibres with serum proteins or bronchial secretions virtually abolishes haemolytic and macrophage surface cytolytic potencies.

Surface cytotoxicity is reflected by rapid killing of cells, as shown by their incapacity to split fluorescein esters within one hour of exposure to particles, and the release of lactate dehydrogenase as well as lysosomal enzymes into the medium.

Confirmation of the importance of interaction with the plasma membrane in early cytotoxicity of chrysotile on macrophages comes from the observation that such cytotoxicity takes place in cells treated with cytochalasin B, in which particle phagocytosis is inhibited.

DELAYED ASBESTOS CYTOTOXICITY

When asbestos particles are added to cultures of macrophages in medium containing serum, they become attached to the plasma membranes of the cells and those of reasonable size are rapidly ingested. Histochemical studies show that lysosomal hydrolases are discharged into the vacuoles containing the particles, so forming secondary lysosomes. Soon after ingestion, serum proteins can be demonstrated on the surface of the particles by immunofluorescence; within two hours of endocytosis, the serum proteins have been entirely digested. The subsequent fate of the cells varies according to the culture conditions and type of asbestos. Allison (1971) and Bey & Harington (1971) found that chrysotile was more toxic than amosite or crocidolite at equivalent concentrations. Some representative results, shown in Table 2, are similar to those reported for hamster macrophages by Bey & Harington.

Table 2. Percentage of mouse peritoneal exudate cells viable[a] after exposure to UICC standard asbestos (100 μg/10^6 cells) for 24 and 48 hours

	24 hours	*48 hours*
Chrysotile	48 ± 7.3	18 ± 6.8
Crocidolite	82 ± 10.4	62 ± 9.7
Amosite	85 ± 7.1	73 ± 8.8

[a] Percentage of cells remaining on coverslips and able to split fluorescein diacetate.

The release of enzymes from cells into the medium paralleled cytotoxicity, but in this case the release of β-glucuronidase occurred before that of lactate dehydrogenase. This supports the conclusion that the initial cytotoxic reaction is with the membrane surrounding the secondary lysosome. Electron micrographs support this view. Soon after endocytosis asbestos particles are confined to secondary lysosomes, but later they are observed throughout the cytoplasm. As previously described, after inges-

tion of chrysotile macrophages frequently show large vacuoles; whereas smaller peritoneal cells (which appear to be a new class of adherent cell), to which asbestos fibres are attached but not engulfed, become intensely basophilic.

When asbestos particles were added to organ cultures of mesothelium, those cells proved to be highly phagocytic, and they, too, showed delayed cytotoxicity in the presence of high doses of chrysotile. However, smaller doses of chrysotile and larger doses of crocidolite were well tolerated. Within 24 hours many vacuoles appeared in the cells, and later these became deep brown in colour, apparently because of the presence of peroxidised lipid pigment. One of the features of cells that have ingested asbestos, in contrast to silica, is that they develop an electron-dense matrix around the particles, and—especially after the ingestion of crocidolite—they synthesise large amounts of ferritin, which accumulates in the secondary lysosomes containing asbestos and so contributes to the formation of asbestos bodies.

Fibroblasts growing from mouse peritoneum alongside mesothelial cells in organ cultures were remarkably resistant to the effects of asbestos. Few particles became attached to them, even when nearby mesothelial cells were densely covered, and particles were rarely, if ever, ingested. However, when asbestos was added to mouse subcutaneous tissue fibroblast cultures in saline medium lacking serum, crocidolite did become attached to the cells and bring about early lysis.

CONCLUSION

There is a large measure of agreement among different workers concerning the major effects of the several types of asbestos on cells in culture. There are two types of cytotoxic effect: an early cytotoxic effect follows soon after asbestos becomes attached to cells in saline media, which is due to interaction with the plasma membrane, and which is markedly inhibited by the presence of serum or other biological macromolecules; a late cytotoxic effect is due to an interaction of ingested asbestos particles with the membranes around secondary lysosomes. Chrysotile and anthophyllite are more active than are crocidolite and amosite in respect of both early and late cytotoxicity. The high magnesium content of the active forms of asbestos probably accounts for their relatively potent interaction with biological membranes. The main interaction may occur with sialoglycoproteins in the membrane which form clusters under the particles and increase permeability to ions and water, with consequent osmotic lysis.

Interactions of asbestos particles with macrophages and mesothelial cells are similar, but there is much less effect on fibroblasts. It seems likely that fibrogenesis is a reaction of fibroblasts which is secondary to the effects of asbestos on macrophages. Since amosite and crocidolite are fibrogenic and only weakly cytotoxic, the macrophage product that stimulates collagen formation is presumably formed as a result of macrophage stimulation rather than damage. The relationship between *in vitro* effects and *in vivo* carcinogenesis is still speculative.

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Experimental methods—organ culture

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Organ culture techniques have been used for metabolic, nutritional, functional and pathological studies. The modern organ culture technique was pioneered by Strangeways & Fell (1926) when cartilage from chick embryos was successfully grown in plasma clot in a culture vessel consisting of a watch glass. The use of a chemically defined medium was first described by Fell & Weiss (1965); this paved the way for biochemical investigations, since in this method the environmental conditions were constant.

Most of the investigations using tissue cultures have been conducted on animal tissue, and the information derived has been extrapolated to apply to humans. This has undoubtedly advanced our knowledge of the basic mechanisms of tissue growth and of differentiation, and it has contributed to the understanding of the pathogenesis of certain diseases. For example, viruses have been successfully maintained in tissues taken from the respiratory tract (Tyrrell & Blamire, 1967; Tyrrell & Bynoe, 1965; Tyrrell & Bynoe, 1966; Tyrrell & Hoorn, 1965) and used in the diagnosis of viral espiratory diseases (Higgins, 1966; Higgins *et al.* 1969; Mostow & Tyrrell, 1972; Roome *et al.*, 1971; Tyrrell & Bynoe, 1966). This has made possible the preparation of various vaccines.

Animal inoculation and inhalation techniques have also proved useful in elucidating the action of various environmental factors, e.g., asbestos (Smith *et al.*, 1965; Wagner, 1962; Wagner & Berry, 1969; Wagner & Skidmore, 1965), glass fibre (Stanton & Wrench, 1972), nickel (Hueper, 1952), silica (Bryson & Bischoff, 1967). However, *in vivo* observations are time-consuming as it is nearly three years before the animals develop a tumour (Wagner & Berry, 1969). It was therefore desirable to develop a more flexible method for these studies; this resulted in the use of the organ culture technique for the investigation of the effects of various fibres on human tissue.

Human embryonic lungs obtained at termination of pregnancy were dissected out, washed in the culture medium BGJ (Biggers *et al.*, 1961) and cut into 2 mm cubes. The culture medium was supplemented with 15% foetal calf serum and 150 μg/ml of fresh ascorbic acid (Rajan, 1969; Rajan & Hopkins, 1970; Rajan *et al.*, 1972). The culture chambers consisted of borosilicate vessels enclosed in a Petri dish. There was a shallow stainless steel platform in each culture vessel, on which the explant rested; 1.5 ml of the medium were added to each of the culture vessels, which were stacked in a Fildes-McKintosh jar and maintained in an atmosphere of 5% CO_2, 50% O_2 and 45% N_2. The medium was changed on alternate days, and all cultures were kept at 37°C in a water-jacketed incubator.

When first explanted, the lungs consisted of a solid mass of tissue (Fig. 1) filled with primitive cells. After 6 days in culture (Fig. 2) the tubes were hollow and there was a single layer of flattened cells lining the lumen of the distinct bronchi. In cultures exposed to a suspension of crocidolite (UICC sample) there was marked proliferation of fibroblasts (Fig. 3) when compared to controls exposed to a normal medium. There was an increased amount of collagen in the interbronchiolar areas. Biochemical investigations (Evans & Rajan) [2] on the spent medium collected at 2-day intervals revealed an increased amount of lysosomal enzymes (Fig. 4) in the crocidolite-treated explants, which was maximal at 2 days. There was also an increased amount of sulphated mucopolysaccharide in crocidolite-treated lungs as evinced by experiments with S^{35} in which foetal lung was grown for one day in $^{35}SO_4$-labelled

[1] MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, UK.

[2] Unpublished data.

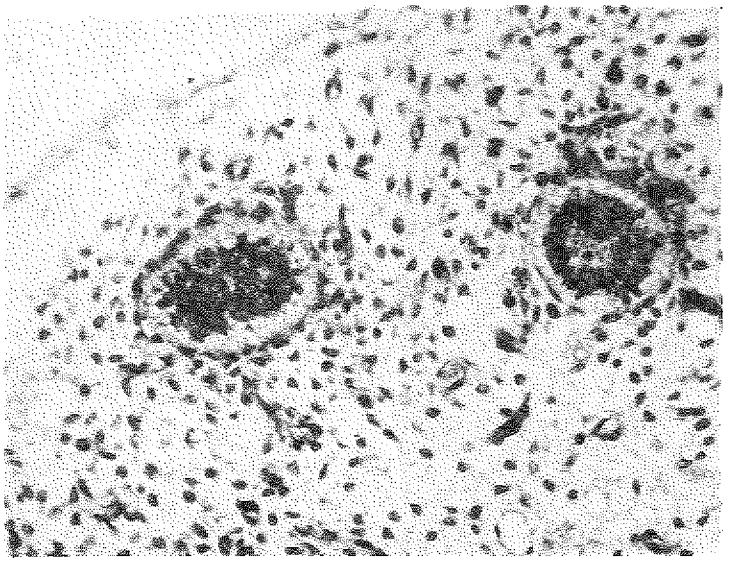

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Fig. 1. Lung from 10-week-old foetus. Note solid bronchioles. Sections stained with haematoxylin and eosin; ×260.

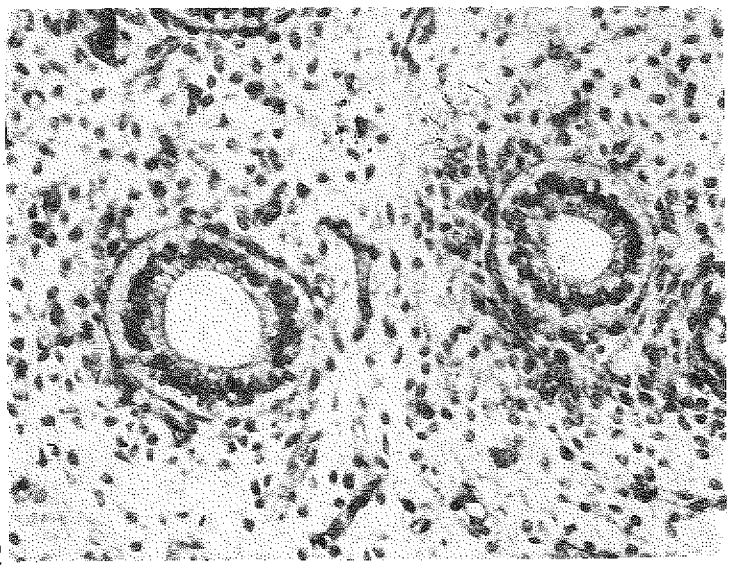

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Fig. 2. Lung from same foetus after 6 days in culture. The bronchiolar lumen is now patent and is lined by a single layer of cells. Sections stained with haematoxylin and eosin; ×260.

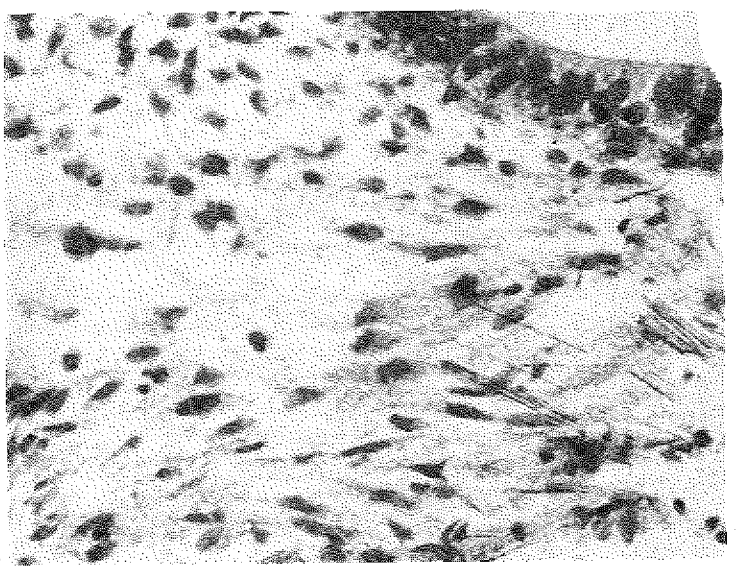

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Fig. 3. Human foetal lung 10 weeks old exposed to a suspension of asbestos (UICC crocidolite) for 6 days. Note the proliferation of fibroblasts. Sections stained with haematoxylin and eosin; ×520.

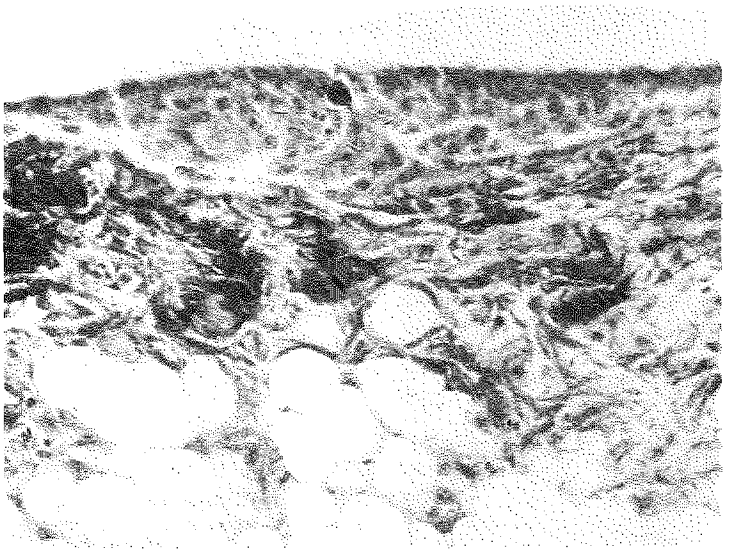

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Fig. 5. Parietal pleura from 50-year-old man, treated with crocidolite for 6 days. There is proliferation of cells of the mesothelial layer. Sections stained with haematoxylin and eosin; ×260.

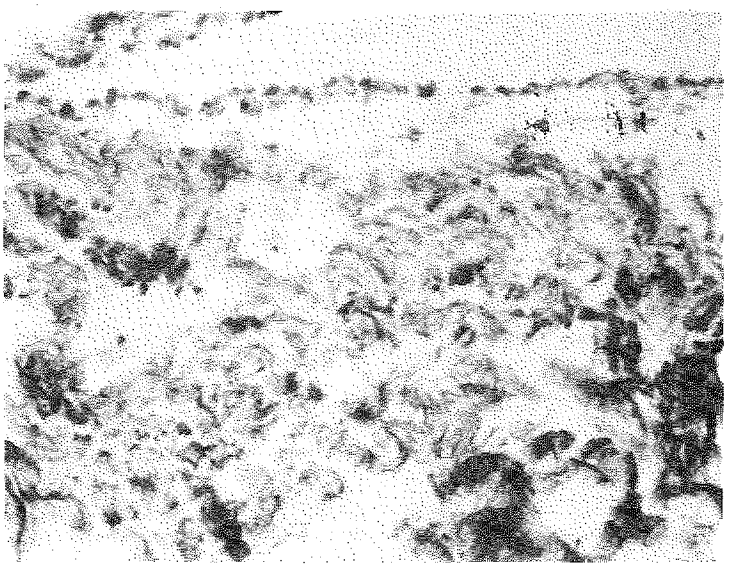

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Fig. 6. Pleura from the same patient in normal medium There is a single layer of mesothelial cells. Sections stained with haematoxylin and eosin; ×260.

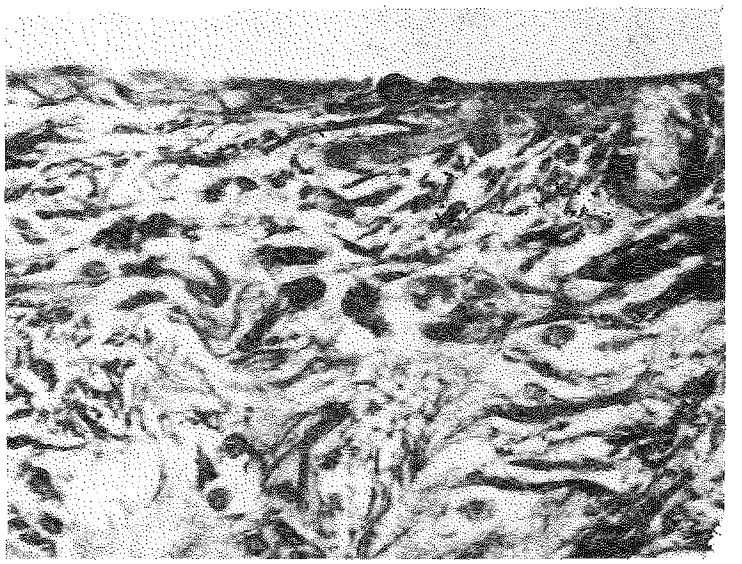

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Fig. 7. Pleura exposed to crocidolite showing invasion of underlying tissue by mesothelial cells. Sections stained with haematoxylin and eosin; ×520.

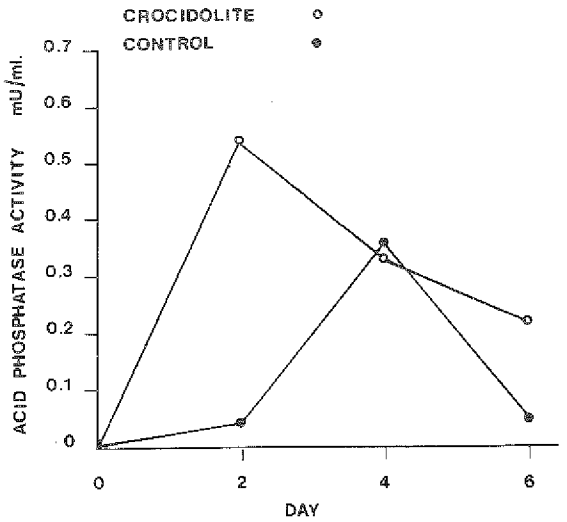

Fig. 4. Acid phosphatase activity, using p-nitrophenyl phosphate as a substrate, determined in the medium at 2-day intervals, in both control and crocidolite-exposed foetal lung organ cultures.

medium, subsequently transferred to unlabelled medium, then exposed to crocidolite fibres for a period of 2 days (Table 1). These experiments indicate that tissues in organ culture respond to crocidolite fibres in a relatively short period.

It was important to test the effects of the dust on adult pleura. Using a similar technique, human parietal pleura obtained at thoracotomy was dissected into 2–5 mm squares and exposed to a suspension of 0.01% crocidolite. A control specimen of pleura was maintained under identical conditions but without asbestos. Results of these experiments indicate a marked proliferation of the mesothelial cells in pleurae exposed to the crocidolite (Fig. 5), as compared to controls in normal medium (Fig. 6). In some areas there was invasion of the underlying tissue by the cells (Fig. 7), and the cells had larger nuclei (7.0 μm SE 0.12) (Fig. 8*a*) than those of the controls (4.5 μm SE 0.11) (Fig. 8*b*).

Table 1. Amounts of sulphated mucopolysaccharide in control and crocidolite-treated explants of human embryonic lungs, as measured by S^{35}

Experiment	% S^{35} remaining in lung [a]		% increased retention
	Crocidolite	Control	
1	12.5	10.8	15.7
2	8.5	7.3	20.5
3a	46.1	40.5	13.8
3b	40.6	34.6	17.3

[a] Radioactivity of both the tissue and the medium was measured using a scintillation counter.

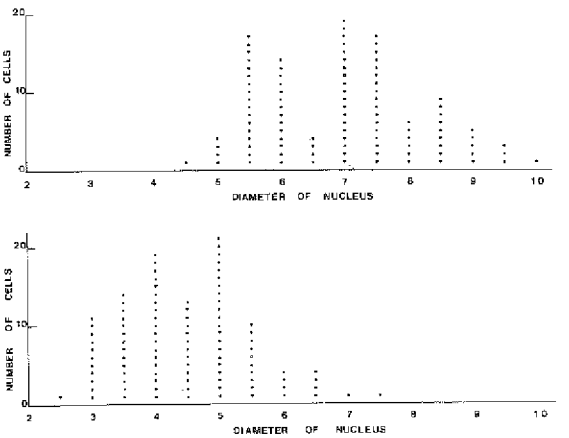

Fig. 8. Distribution of the size of the nucleus in cells (*a*) treated with crocidolite and (*b*) maintained in normal growth medium. Note the shift to the right in treated explants due to larger nuclei.

These experiments demonstrate that human adult tissue will not only survive in organ culture for a period of 6–8 days but will continue to respond to stimuli even when devoid of its blood supply. Therefore, this relatively simple culture system should provide valuable material for investigations of the morphological and biochemical results of various physiological and pathological conditions.

SUMMARY

Human embryonic lungs and adult pleura have been maintained in organ culture, using a synthetic medium containing serum, for periods of up to 10 days. The response of these organs to asbestos has been investigated. Embryonic lungs exposed to crocidolite show proliferation of fibroblasts and mesothelial cells. Similar results were obtained with adult pleura, the additional features being areas showing invasion by cells of the underlying connective tissue.

Biochemical investigations showed enhanced release of lysosomal enzymes in treated explants. Increased amounts of sulphated mucopolysaccharides were retained in crocidolite-exposed lungs, as evinced by S^{35} uptake experiments.

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Some results of experimental studies in asbestos carcinogenesis

L. N. PYLEV [1] & L. M. SHABAD [1]

To study the possible carcinogenicity of asbestos, as well as the significance of benzo(a)pyrene (BP) in asbestos carcinogenesis, experiments have been undertaken in non-inbred rats and morphological studies have been made of the lesions caused by asbestos.

Samples of chrysotile were obtained from two different mines in order to study their relative BP contamination. Chrysotile from the first mine (chrysotile I) differed from that from the second one (chrysotile II) in that it was of a higher quality and possessed longer fibres. The asbestos samples were ground, if necessary, prior to investigation.

The samples were extracted in cold benzene for 12 to 16 hours and were then subjected to hot extraction in the same benzene for 8 to 12 hours. We studied the extract on a DSP-51 spectrograph by the technique developed in our laboratory by Fedoseeva & Khesina (1968). The results are presented in Table 1.

BP was found in all the samples investigated, a greater amount of it being found in chrysotile I. It is worth mentioning that a certain amount of BP (9.96 μg/kg) was also found in asbestos samples obtained directly from the mine. The greatest amount of BP was found in commercially produced asbestos (36.9 μg/kg and 16.00 μg/kg); this may be due to its high adsorption properties which allow the asbestos to adsorb BP present in the environment.

In order to study this point further we have investigated the adsorption properties of chrysotile dust collected in a factory within the workers' breathing zone. Two grams of asbestos were placed in BP-

Table 1. Amount of BP in chrysotile samples

	Chrysotile I	Chrysotile II
	Mean amount of BP μg/kg ± S.D.	Mean amount of BP μg/kg ± S.D.
Ore from mine	9.96 ± 2.08	not studied
Ore before drying	3.10	2.80
Ore after drying	3.80	2.50
Fifth stage of concentration	5.70 ± 1.48	not studied
Sixth stage of concentration	5.40 ± 2.67	2.39 ± 1.41
Commercial asbestos	36.90 ± 0.66	16.00 ± 2.57
Asbestos dust from working place	11.70 ± 2.31	3.92 ± 1.32

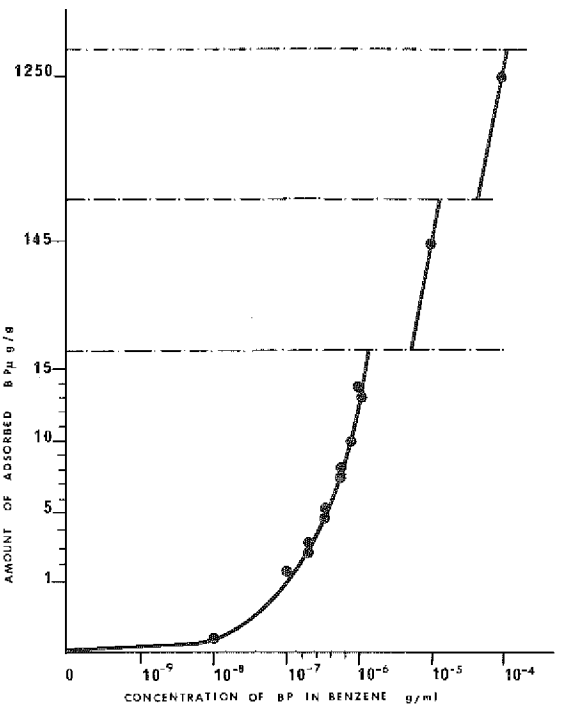

Fig. 1. Adsorption of BP from benzene by asbestos.

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benzene solutions of various concentrations and kept at room temperature for three days, with periodic shaking; then the residual concentration of BP in the solution was determined. The results are presented in Figure 1. Notice that the amount of BP adsorbed per gram of dust greatly increases with the increase of BP concentration in benzene. Thus, at a concentration of 1.10^{-5} g/ml, 1 gram of dust adsorbs 145 μg of BP; while at a concentration of 1.10^{-4} g/ml the BP adsorbed amounts to 1250 μg. A comparison of the amount of BP present in the dust with that found in the adsorption experiments suggests that the ability of the dust to adsorb BP had not yet been exhausted. Thus, as the concentration of BP in the environment increases, the asbestos dust will continue to adsorb it.

For a series of experiments *in vivo*, chrysotile I was ground (70% of the fibre ranged from 5 to 15 μm in length) and was extracted in hot benzene. Various concentrations, suspended in 0.2 ml of saline, were introduced intratracheally into the rat lung.

Four series were undertaken in this experiment; rats were treated as follows:

(1) Three applications of 2 mg of dust at monthly intervals.

(2) Three applications of 2 mg of dust together with adsorbed BP (the total amount of BP adsorbed was 0.144 mg), at monthly intervals.

(3) One application of a mixture of 2 mg of dust and 5 mg of BP.

(4) One application of 5 mg of BP.

The results of these investigations are shown in Table 2.

Previously published data concerning the effects of one intratracheal injection of 0.6 mg of BP into rats (Pylev, 1969) were used as a control to series 2; this dose of BP induced neither lung tumours nor precancerous lesions.

In a second experiment, rats were given three intrapleural injections of 20 mg of chrysotile I dust in 0.5 ml of saline at monthly intervals. The dust was obtained at a factory, within the workers' breathing

Table 2. Morphological lesions in rat lungs induced by intratracheal injections

	Chrysotile 2 mg three times		Chrysotile 2 mg with adsorbed BP three times		Chrysotile 2 mg + 5 mg of BP		5 mg of BP	
Survival time	1–6 weeks	9–28 months	1–14 weeks	10.5–25.5 months	1–6 weeks	9–28 months	1–6 weeks	9–28 months
	Number of animals							
Morphological lesions	12	49	30	21	24	11	9	19
Diffuse irregular hyperplasia of alveolar epithelium	—	—	—	—	1	—	—	2
Focal growth of alveolar epithelium	—	—	—	1	—	1	—	—
Diffuse irregular hyperplasia of bronchial epithelium	—	—	1	2	—	—	—	—
Focal growth of bronchial epithelium without squamous metaplasia	—	9	9	15	9	5	1	2
Focal growth of bronchial epithelium with squamous metaplasia	—	6	5	7	19	8	1	3
Focal adenomatous growth of bronchial epithelium and bronchial mucous glands	—	3	—	3	—	—	—	2
Lung papilloma	—	—	—	2	—	1	—	—
Epidermoid carcinoma	—	—	—	—	—	3	—	—
Reticulosarcoma	—	—	—	4	—	—	—	—
Diffuse irregular hyperplasia of pleural mesothelium	—	—	2	3	—	—	—	—
Focal hyperplasia of pleural mesothelium	—	3	4	8	1	4	—	—
Focal adenomatous growth of pleural mesothelium	—	—	—	1	—	1	—	—
Pleural mesothelioma	—	—	—	—	—	2	—	—
Total number of tumours		0		6 (28.6%)		6 (54.5%)		0

zone. 80% of the fibres ranged in length from 1 to 30 μm. The results of this investigation are presented in Table 3. Some of the animals are still alive.

Table 3. Morphological lesions in rat pleural mesothelium induced by intrapleural injections of chrysotile (20 mg three times)

Survival time (months)	2–2.5	8–18.5
	Number of animals	
Morphological lesions	3	48
Diffuse irregular hyperplasia	2	4
Focal hyperplasia	3	36
Focal adenomatous growth	2	7
Sarcoma-like mesothelioma	–	11
Tubo-papillary form of mesothelioma	–	1
Mixed form of mesothelioma	–	6
Lung adenoma	1	1
Lung reticulosarcoma	–	2
Total number of mesotheliomas	0	18 (37.5%)

RESULTS AND CONCLUSIONS

From the data presented in Table 2 it is evident that when the rats were treated with pure asbestos only, an insignificant number of the animals developed precancerous lung lesions; these were observed only later in the experiment. No tumours were found. Analogous injections of asbestos together with adsorbed BP (6 mg asbestos and 0.144 mg BP) resulted in a great number of precancerous lung lesions. These were observed within the first weeks of the experiment and developed maximally at the end of it. Their morphology did not differ from that described by us previously (Pylev, 1962; Shabad, 1962, 1967; Shabad & Pylev, 1970). No lung cancer was found in this series of the experiment, but a number of animals developed lung papillomas and reticulosarcomas. Pulmonary tumours were found in 28.6% of the animals.

In the third series of this experiment the rats that received one injection of 2 mg of dust with 5 mg of BP also developed precancerous lung lesions. A lung papilloma was found in one rat, lung cancer in three, and mesotheliomas in two rats. Tumours were observed in six animals out of the eleven (54.5%) which died at the end of the experiment.

In the fourth series of the experiment (one injection of 5 mg of BP) no lung tumours were found. Some of the rats developed precancerous lesions.

Besides the precancerous lesions found in the lungs of animals in series 1, 2 and 3 of the experiment, pleural mesothelium lesions, which we classify as premesotheliomatous growths, were also found.

Thus, tumours were found in those series in which rats were given injections of asbestos together with BP. It may be that the asbestos retards the removal of BP from the lungs and in this way contributes to the development of neoplastic growth. This is in agreement with our previous finding (Pylev *et al.*, 1969) that asbestos can retard the removal of BP from hamster lung.

When chrysotile was administered intrapleurally (Table 3), pleural mesotheliomas were found in 18 animals out of the 48 (37.5%) which died; lesions were first observed eight months after the beginning of the experiment. One of the tumours was successfully transplanted subcutaneously to standard rats, and by September 1972 it had undergone eleven generations.

The morphology of these mesotheliomas did not differ significantly from that of the tumours described by Wagner (1962; 1970) and Wagner & Berry (1969). The tumours most frequently found had a sarcoma-like structure (Fig. 2). In a number of tumours areas of both sarcoma-like and of adenomatous structure could be observed; we regard these as mixed mesotheliomas (Fig. 3). A pleural mesothelioma of a purely adenomatous structure was found in only one case (Fig. 4).

Of great interest are the lesions which we found in the pleural mesothelium. In Shabad's classification of precancerous stages they would be described as pre-tumorous, i.e., premesotheliomatous growths. Up to now no such findings have been reported in the literature; we should therefore like to draw attention to some details.

A number of animals developed diffuse irregular hyperplasia of the pleural mesothelium; vast areas of the lung surface were covered with one layer of large cuboidal cells having basophilic cytoplasm and hyper- or hypochromatic nuclei (Fig. 5). This growth originated both with a background of insignificant pleural sclerosis and without it. More frequently, the development of focal hyperplasia was observed, with proliferations of mesothelial cells which occurred both in the presence and absence of diffuse hyperplasia of the mesothelium. One characteristic of these proliferations was the appearance of cell accumulations in the form of pillow-like and polypus-like outgrowths on the lung surface. The cells of cuboidal and oval forms were located at ran-

dom in several layers (Figs. 6 & 7). These lesions could be seen both inside and around asbestos granulomas (Fig. 8).

In a number of cases these focal outgrowths were of adenomatous structure and protruded either on the pleural surface or were located under it (Fig. 9).

As a rule, the focal lesions of the mesothelium described arose multicentrically; in a number of cases the same animal developed both various types of premesotheliomatous growth and pleural tumours.

Precancerous lesions have been described in a study of carcinogenesis in the skin, lung, liver, kidney, stomach and other organs (Shabad, 1967).

The premesotheliomatous pleural lesions which we have now found indicate again that each tumour develops in accordance with general laws: first we observe the development of diffuse irregular hyperplasia; then a focal hyperplasia is seen, followed by the development first of benign and then of malignant tumours. Further investigation of premesotheliomatous lesions may enable us, as in our study of tumours in other organs and localisations, to investigate the mechanism of the blastomogenic action of asbestos and to outline ways of prevention and treatment of pleural tumours.

Two of the rats which developed pleural mesotheliomas also developed lung adenomas originating in the bronchial epithelium; and two more rats developed reticulosarcomas which arose in lymphoidal tissue around the bronchi and vessels.

SUMMARY

The carcinogenic activity of various types of asbestos from different parts of the world has been studied experimentally in animals (Schmähl, 1958; Wagner, 1962, 1970; Wagner & Berry, 1969; Smith *et al.*, 1965, 1968, 1970). In these reports not only various types of asbestos, but also samples of the same type of asbestos extracted from different mines were investigated. Thus, according to Wagner (1970), different carcinogenic activity was observed in the same type of asbestos obtained from different mines. However, until recently there has been no data concerning the possible blastomogenicity of the asbestos found in the Soviet Union.

The mechanism of the blastomogenic activity of asbestos is obscure. Among others, the suggestion has been made that the carcinogenicity of asbestos is due to its contamination by other materials, and specifically by carcinogenic polycyclic aromatic hydrocarbons.

According to the findings of Harington (1962; 1965), some mine samples of certain types of asbestos may contain benzo(a)pyrene (BP), which may also contaminate the asbestos during transportation and storage. We [1] have therefore investigated BP contamination of various types of asbestos found in the Soviet Union.

[1] In collaboration with L. V. Krivosheeva.

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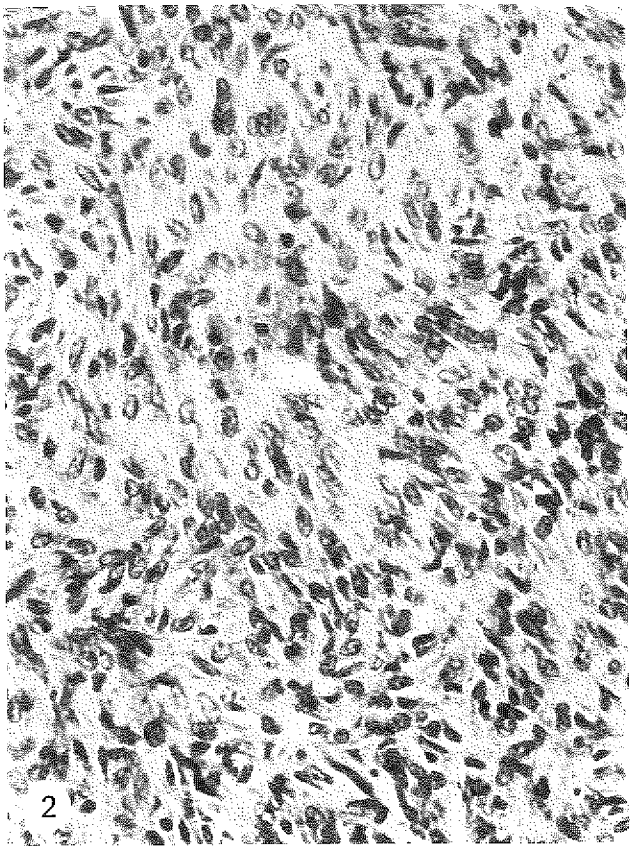
Fig. 2. Sarcoma-like pleural mesothelioma.

Fig. 3. Mixed form of pleural mesothelioma. Foci of sarcomatous and adenomatous structure.

Fig. 4. Tubo-papillary form of pleural mesothelioma.

Fig. 5. Diffuse irregular hyperplasia of pleural mesothelial cells.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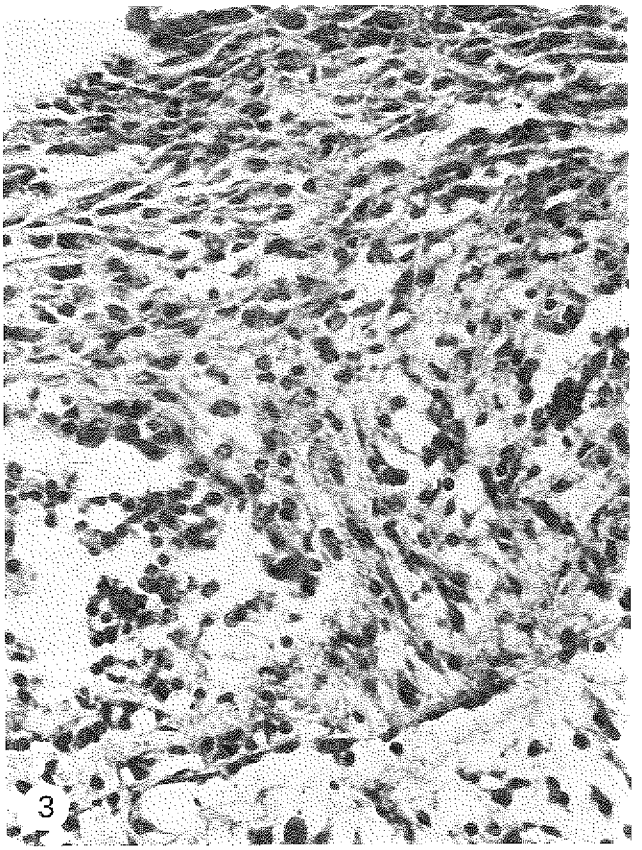
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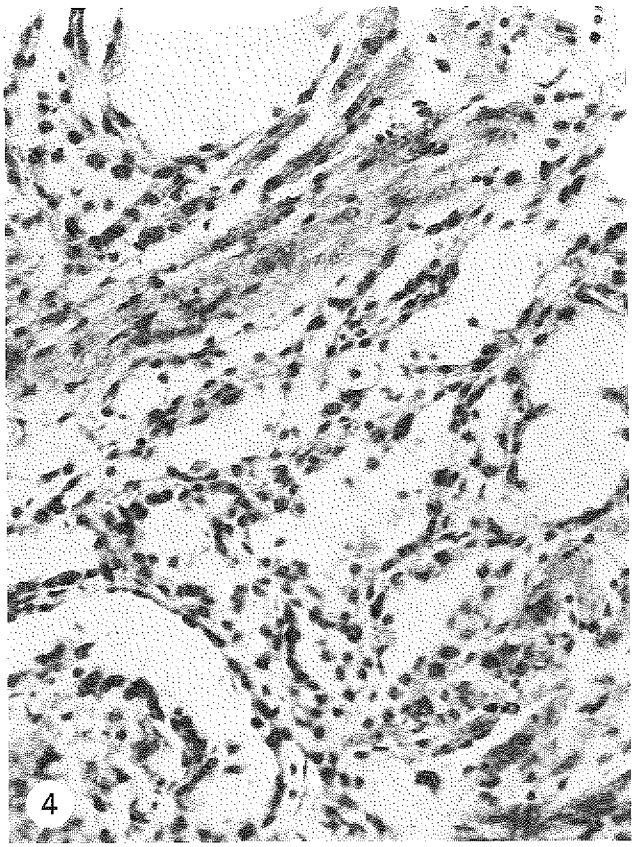
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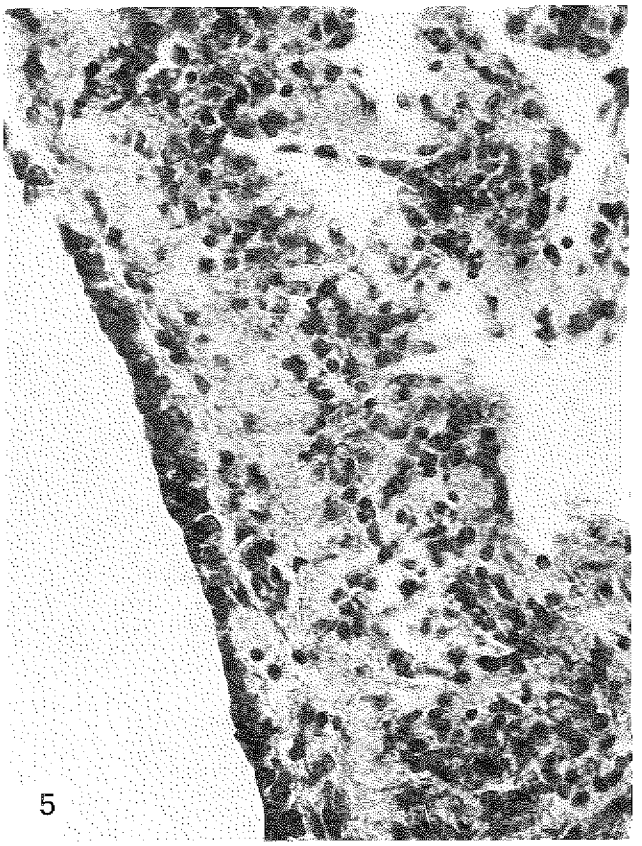
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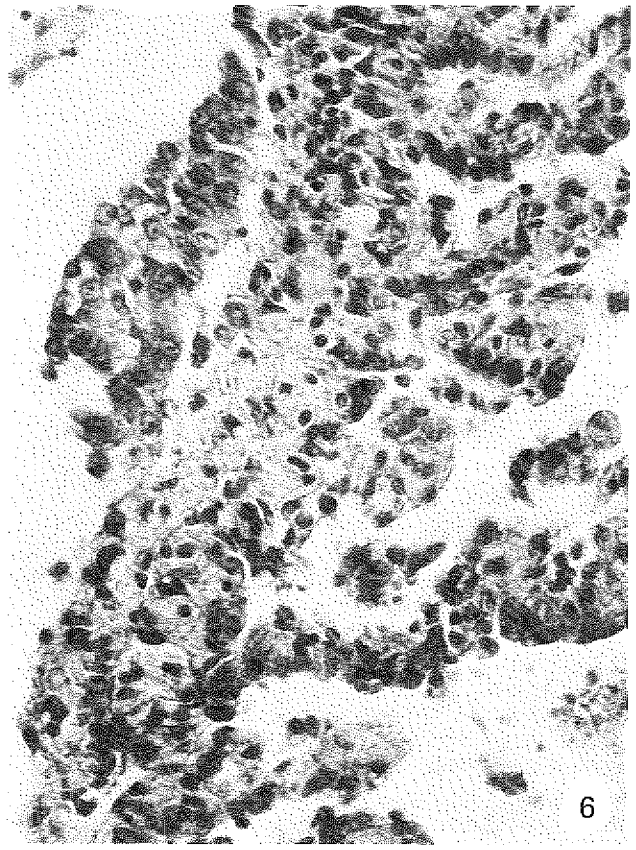
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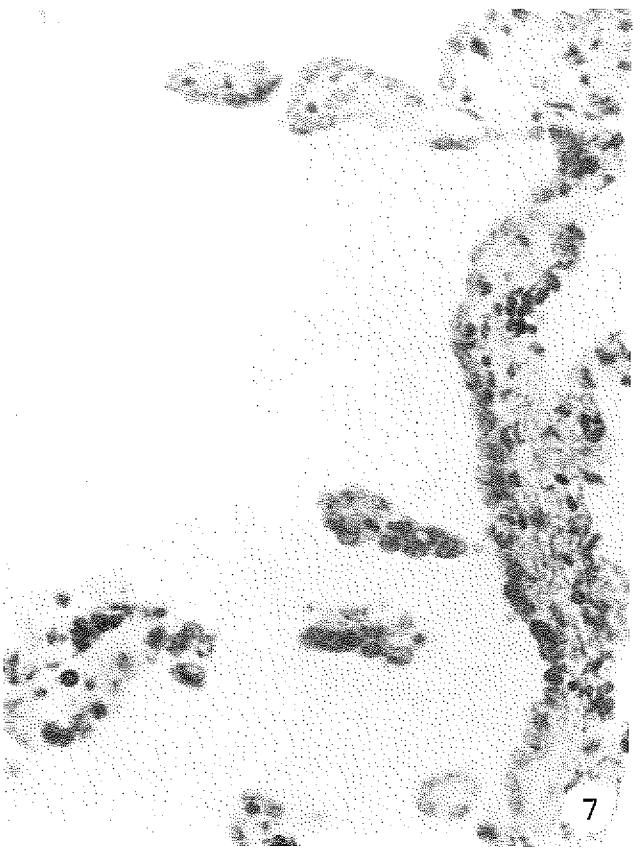
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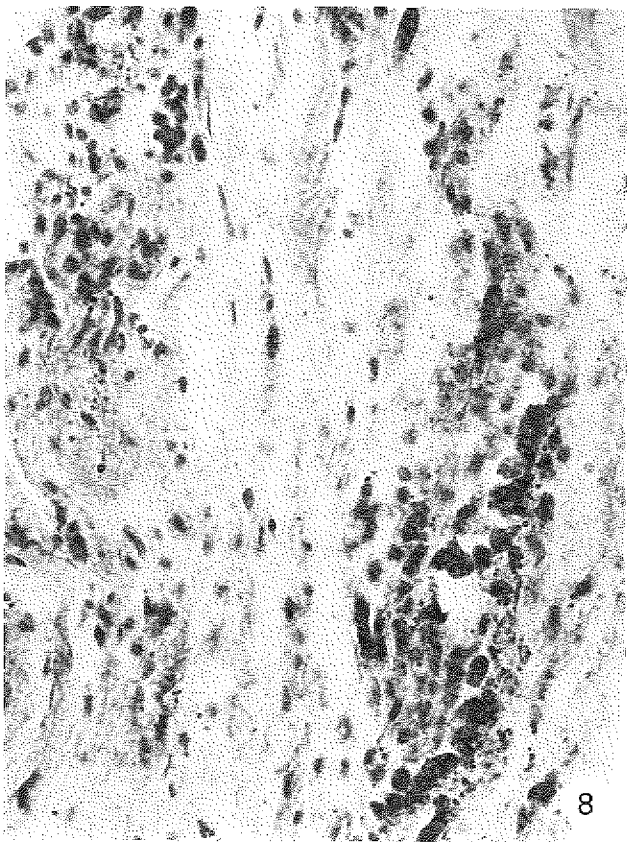
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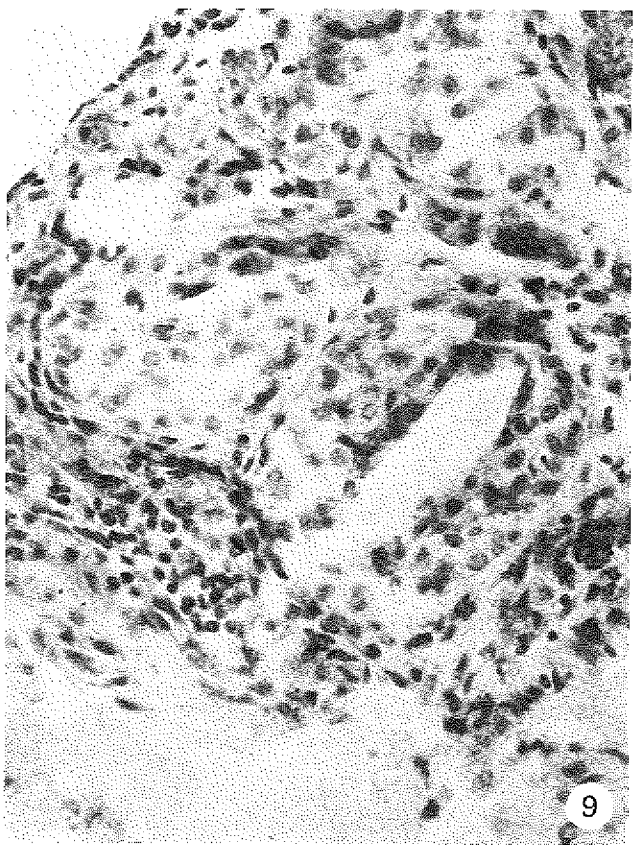
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Fig. 6. Focal hyperplasia of pleural mesothelial cells in the form of pillow-like outgrowths.

Fig. 7. Focal hyperplasia of pleural mesothelial cells in the form of polypus-like outgrowths.

Fig. 8. Focal hyperplasia of pleural mesothelial cells inside asbestos granuloma.

Fig. 9. Focal adenomatous outgrowths of pleural mesothelial cells.

Discussion summary

J. S. HARINGTON [1]

INVESTIGATIONS USING ANIMALS

A claim was made that the baboon is an ideal experimental animal for the inhalation technique in that it resembles man in several respects. It is large enough for biopsies and radiological and cardiopulmonary tests to be carried out. The lesion produced in the baboon by the inhalation of asbestos does not appear to progress if the animals are removed from the dust. In view of this lack of progression, baboons should be exposed throughout the entire experiment. Bronchio-alveolar tumours had developed in baboons exposed to UICC crocidolite. Mention was made that a mesothelioma had been induced in a monkey following intrapleural inoculation of milled country rock from the crocidolite area in the north-western Cape of South Africa, but the amount of fibre in the dust was not given.

On co-carcinogenesis, it was stated that the combined effect of benzo(a)pyrene and chrysotile was greater than that of either agent administered singly. The hydrocarbon was effective as a carcinogen at much lower dosages when given with chrysotile. Adsorption of benzo(a)pyrene on chrysotile could have some effect on the carcinogenicity of the asbestos.

The use of insect larvae as a means of distinguishing the effects of different forms of asbestos was described. Among other effects, changes in haemolymph cells were seen.

EXPERIMENTAL METHODS—ORGAN CULTURE

Attention was drawn to the existence of several types of cells in organ culture, depending on whether embryonic or adult tissue is used. With embryonic tissue there is a pleomorphic response, whereas with adult pleura it is predominantly a mesothelial and fibroblastic proliferation. Glass fibre of diameter and length similar to crocidolite had been used in these cultures. In embryonic lungs an intensive fibrotic reaction was produced with numerous cells containing ingested glass, and in certain areas giant cells containing up to six nuclei were seen. A hyperplastic proliferation of mesothelial cells also occurred. Alterations in the relative proportions of mucopolysaccharides, depending upon age of tissue had been noted. A higher proportion of hyaluronic acid to chondroitin sulphate was found in embryonic cultures than in matured tissue. The increased amount of hyaluronic acid in pleural fluid from patients with mesothelioma may indicate transformation from mature to primitive cells. The presence of a raised amount of this polysaccharide in the pleural fluid may help in early detection of cell transformation.

EXPERIMENTAL METHODS—CELL AND TISSUE CULTURE

It was said that there is no evidence of any interaction between lymphocytes and asbestos fibre in the *in vitro* system used. No immuno-competent cells were present. It was mentioned that the sera modified the toxicity of asbestos to macrophages (and fibroblasts). The activities of certain forms of asbestos could also be changed considerably by heating, and conceivably by type of milling or preparation of fibre. The inhibition of incorporation of thymidine into 3T6 fibroblasts by asbestos was described. Smaller amounts of asbestos accelerate incorporation. Quartz silica inhibits proliferation of these cells.

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ASSESSMENTS OF METHODS USED IN THE STUDIES OF THE BIOLOGICAL EFFECTS OF ASBESTOS. 4. ENVIRONMENTAL

Chairman — W. H. Walton

Rapporteur — V. Timbrell

Sampling methods

S. HOLMES [1]

In recent years considerable progress has been made in the sampling and analysis of airborne dusts; thus some correlation can be made between inhalation of dust and its biological effects, and more reliable data can be obtained for improving dust control in factory atmospheres. The introduction of sampling instruments which collect only the respirable fraction of a dust cloud has made possible the evaluation of samples by gravimetric or mass estimation, a process which for most particulates is simple and reproducible. Furthermore, the long period sampling generally necessary for mass estimation gives a measure of average exposure which is generally of more relevance in assessing a hazard than are measurements of peak concentrations.

THE ASBESTOS PROBLEM

Because the respirability characteristics of compact, near-spherical particles are well established, it is possible to design pre-filtering or elutriation systems which will ensure that only the respirable fraction of a dust cloud is collected in the instrument. Fibrous dusts, however, present special problems. The respirability of all dust particles is determined by their free-falling speed in air, which in the case of fibres is related to their diameter; but the degree to which they are retained in the human lung is dependent upon the length of the fibre. Thus, the lung clearance mechanisms will remove more of the respirable fraction if it is in the form of short fibres than would be the case for the same mass of long fibres, the diameter being assumed constant. It has been suggested (Timbrell & Holmes, 1970) that even if an effective elutriator could be devised, an estimation of the mass of fibre retained in the lung as derived from the respirable mass collected by the sampling device would be of doubtful reliability.

The use of a pre-filtering system for asbestos fibres presents several problems. Since their site of deposition in the respiratory tract, should they reach the fine airways, depends on their length and shape, the difference between the straight shape of amphibole fibres (amosite, crocidolite and anthophyllite) and the "curly" nature of chrysotile becomes important. This variation between chrysotile and the amphiboles has been reported by Timbrell (1970), who has demonstrated that curvature in a fibre tends to reduce the efficiency of penetration and to cause the fibre to be deposited higher in the respiratory tract. Thus, a satisfactory pre-filtering system for asbestos should take account of the effect of shape on the respirability of the fibres. Horizontal elutriators, which have been successfully used to separate particles of compact shape, filter solely on the basis of particle falling speed and are insensitive to the length or shape of fibres (Timbrell, 1972). The use of a device such as a bundle of capillary tubes would be helpful; and since the samples required for microscope counting are small there would be no necessity for cleaning the device during the sampling period. A pre-filter capable of providing a constant performance over the time necessary to collect a weighable sample is not available.

OCCUPATIONAL SAMPLING OF ASBESTOS

Since the probable importance of the longer fibres in the fibrogenic effects of asbestos has been recognised for some considerable time (Vorwald *et al.*, 1951), the asbestos industry has tended to concentrate on methods for obtaining samples suitable for microscopic analysis, in which the long fibres could be recognised and classified. In the search for a suit-

[1] Asbestosis Research Council, Turner Brothers Asbestos Company Limited, Rochdale, Lancashire, UK.

able instrument, all the dust samplers available at the time have been tried and discarded for one reason or another (Holmes, 1965). Instruments based on impingement tended to break up the fibre bundles; and those based of thermal precipitation (except the long-running precipitator) appeared to be very inefficient collectors of the longer fibres. Eventually, the membrane filter method was chosen as the most likely system of sampling for asbestos fibres, and development of this method proceeded both in the UK and the USA.

The principle of the method is that the dust sample is collected on a cellulose membrane filter of fine pore size. The filter is subsequently mounted on a microscope slide and cleared (i.e., rendered transparent) with a liquid whose refractive index is such that the contrast between the fibres and the background is not impaired. The sample is then counted at a magnification of about 500×, and to further enhance the visibility of the fibres a phase contrast microscope is used.

For the purpose of analysis, a particle is defined as a fibre if its length is at least three times its diameter and if it is at least 5 micrometres in length. In addition, in the UK it is customary to set 3 μm as an upper limit on the diameter, on the grounds that fibres of larger diameter than this would not be respirable. The method has the great advantage that asbestos fibres are easily recognised and this is essential where mixed dust is involved. The lower length limit of 5 μm was chosen somewhat arbitrarily in the early days of the development of the method; however it is significant that fibres which are retained for long periods, either free or as the cores of asbestos bodies, in the lungs of occupationally exposed persons are generally longer than this.

The Dust Measurement Working Group of the Asbestosis Sub-Committee of the Permanent Commission has recommended the membrane filter method for acceptance as the standard method for assessing occupational exposure to asbestos, and measurements based on the method were used by the British Occupational Hygiene Society (1968) for the formulation of its hygiene standard for chrysotile asbestos dust. Government controls on asbestos dust emission in both the UK and USA are also expressed in terms of fibre counts, to be measured in the same way.

The membrane filter method possesses one important disadvantage in that the mounting and counting of the sample is a somewhat tedious operation. Means of speeding-up the work have been sought, and over the last few years the UK industry has made good use of an American instrument, the Royco Automatic Particle Counter, for routine measurements in situations such as textile factories where the dust is almost entirely asbestos. This has been made possible because, while the Royco instrument depends for its functioning on light-scattering and therefore "sees" all dust particles which pass through it, its size-discrimination facility can be adjusted to give the same numerical reading as would be obtained from a membrane filter sample taken in the same place and at the same time. It is also fitted with an automatic print-out device, thus the necessary information is provided simply and quickly without the tedium of mounting the sample and counting fibres under the microscope.

While the Royco continues to be used for routine factory monitoring and engineering controls, it is not suitable for biological studies, in which personal sampling is preferable; for this the membrane filter method is ideal, being extremely portable. Epidemiological studies already planned will involve the collection and analysis of large numbers of membrane samples, and this has led to a search for an instrument which will automatically count and size-select fibres directly from the microscope slide. Among a number of instruments already examined, the Quantimet 720 (IMANCO Ltd) is showing particular promise. Using a phase contrast microscope system with automatic focusing, the Quantimet should be able to deal with an automatically fed magazine load of microscope slides. As might be expected, the cost of such a system is likely to be considerable and could only be justified where large numbers of samples are involved.

The membrane filter method can be criticised on the grounds that very fine asbestos fibres may not be seen in the light microscope, even under the best viewing conditions. The investigation of alternative methods of analysis, usually based on mass estimation, is not being neglected, in spite of the difficulties associated with asbestos referred to above. Methods so far tried include atomic absorption spectroscopy (by which the magnesium in chrysotile can be estimated) and infra-red analysis. The most promising method explored so far, however, is based on X-ray diffraction, where even in a mixed dust sample the asbestos can be specifically identified by its crystal structure. Using an X-ray diffractometer it is possible to measure as little as 10 micrograms of chryso-

tile asbestos in a sample where the asbestos represents perhaps only 10% of the total mass.

ENVIRONMENTAL SAMPLING OF ASBESTOS

In recent years there has been considerable speculation concerning asbestos as a public health hazard in view of its widespread usage in a variety of forms, including brake linings, in spite of the fact that for the latter application it has been shown that due to heat degradation the wear products which are generated contain little fibrous asbestos. The emission of dust from plants manufacturing asbestos products has also been mentioned as a potentially dangerous source of pollution.

The asbestos industry in the UK has therefore felt obliged to investigate the position, and it has instituted a programme of measurements in the vicinity of its factories, and in selected urban and rural situations. It was quickly demonstrated that asbestos, if it was present at all, existed in such small quantities that the standard sampling instruments and methods were completely inadequate. To obtain any measurable quantity of asbestos, it was obviously going to be necessary to sample very large volumes of air, and if this was to be collected in a reasonable time, a high speed sampling instrument would be necessary. Such an instrument is the Litton Sampler (Rickards & Badami, 1971) which works on the principle of electrostatic precipitation and is capable of sampling speeds of up to 10,000 litres of air per minute. This means that sample volumes of a million litres (1000 cubic metres) can be collected in a reasonable time.

Even in these large volume samples, estimation of the asbestos load has proved difficult. Where the total mass is above about 100 micrograms (i.e., 10^{-7} grams per cubic metre) the X-ray diffraction method has been used; but in many of the samples collected, no asbestos has been detected by this method. In these cases, it has been necessary to treat the sample by ultra-sound to disperse any asbestos present into its basic fibril form and to estimate the mass using transmission electron microscopy. This method, which is shortly to be published, can measure asbestos concentrations as low as 10^{-10} grams per cubic metre in a 1000 cubic metre sample, even in the presence of excessive amounts of other airborne solids.

To sum up, the combination of membrane filter sampling and optical microscope analysis is still considered to be the best system for monitoring asbestos dust in occupational situations. Mass estimation methods have the great advantage of rapidity, but they cannot be adopted with confidence, particularly for chrysotile, until a reliable elutriation system is available. Furthermore, because of the belief that the longer fibres may be of special significance as regards a health hazard, a technique for sizing and counting of fibres must be considered important.

On the other hand, for assessing the asbestos content of general environmental atmospheres mass estimation is the only available method. In this type of study the question of elutriation does not arise, since it can be confidently assumed that all the fibres which remain airborne in these circumstances will be respirable. The concentrations of asbestos seen under these conditions are so small that large volumes of air have to be sampled; however, here high-speed samplers can be used, which is not the case for occupational situations. The fibres collected are generally too fine to be seen under the light microscope, particularly in the presence of a high concentration of other atmospheric pollutants. Estimation of the asbestos content has therefore to be made on a mass basis using X-ray diffraction or electron microscopy, depending upon the quantity collected in the sample.

SUMMARY

The special problems associated with the sampling and analysis of fibrous dusts are discussed. The merits and disadvantages of sampling methods based on fibre counting and mass determination are reviewed in relation to sampling in both occupational and general environmental situations.

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Chemical characteristics of asbestos and associated trace elements

A. MORGAN [1] & L. J. CRALLEY [2]

The known varieties of asbestiform minerals can be classified either as serpentine or amphibole according to their crystal structure. Chrysotile is the sole commercial representative of the serpentine class and is by far the most common of the asbestiform minerals, accounting for over 90% of the asbestos produced today. The five amphibole varieties used commerically are amosite, anthophyllite, crocidolite, actinolite and tremolite. The asbestiform minerals have been the subject of a number of reviews in recent years (Hodgson, 1965; Speil & Leineweber, 1969). Their physical and chemical properties can be related directly to their crystal structures, which have been well established.

In addition to its major structural elements, asbestos invariably contains trace metal impurities. These may be present as isomorphous substitutes for structural elements, as fragments of host rock and accessory minerals; or as fine particles of alloy produced by the abrasive action of the asbestos on processing equipment (Cralley *et al.*, 1967). This paper will review current information concerning the levels of designated trace metals in asbestos with particular reference to the UICC standard reference samples.

CHRYSOTILE

Composition

Chrysotile ($Mg_3Si_2O_5(OH)_4$) has a layered structure, the basis of which is a sheet of silica tetrahedra. Attached to one side of this is a brucite ($Mg(OH)_2$) layer, in which two out of every three hydroxyls are replaced by the apical oxygens of the silica tetrahedra. A mismatch in the dimensions of the two sheets introduces a strain into the structure; this is relieved by curvature, resulting in the hollow cylindrical morphology of the chrysotile fibril. Fibril diameters vary between about 10 and 80 nm, but the mean values are generally in the range of 30–40 nm (Atkinson *et al.*, 1971). Chrysotile fibres consist of bundles of fibrils, and it has been suggested that the voids between the bundles are filled with a non-crystalline solid.

Associated trace elements

The minerals most commonly found in association with chrysotile are magnetite (Fe_3O_4) and brucite, which may be intergrown with the fibre. Gibbs (1971) has reported that other minerals associated with chrysotile from the asbestos mining areas in Quebec include actinolite, antigorite, awaruite, chlorite, chromite, magnesite, nemalite and talc.

Magnetite can be separated magnetically from fully opened fibre. Using neutron-activated samples, Morgan *et al.* (1971b) separated magnetic fractions from a number of samples of chrysotile, and they found that the fraction of iron present as magnetite varied from 19–58%. Small amounts of chromium (3–13%) and cobalt (7–23%) were separated with the magnetic phase, but in no case was any of the scandium removed by this method.

Neutron activation analysis (NAA) has been used by Holmes *et al.* (1971) to measure the levels of chromium, cobalt, iron, manganese and scandium in a number of samples of asbestos, including the UICC standard reference samples. These values, together with measurements of nickel by atomic absorption spectrophotometry (AAS) are sum-

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marised in Table 1. Also included in Table 1 are measurements made on the eight samples of Canadian chrysotile which were blended to produce the UICC sample of Canadian chrysotile (Chrysotile B). Cralley *et al.* (1968) used AAS to measure a similar range of elements in the UICC samples and their values are also included in Table 1. With the exception of iron, agreement between the two laboratories is satisfactory.

In general, nickel is best determined by AAS and is homogeneous at the 10 mg level, using the reproducibility of replicate determinations of a number of trace metals as an index of homogeneity. Another advantage of NAA is that it can be used non-destructively. To achieve accurate results with AAS, it is essential to ensure that both fibre and accessory minerals are completely dissolved before the solution is aspirated. Some minerals are insoluble in boiling hydrofluoric acid, and it is necessary to use fusion to dissolve all the contaminating minerals.

Table 1. Levels of trace metals in samples of asbestos from various regions

Source	Lab	Fe (%)	Cr (ppm)	Co (ppm)	Mn (ppm)	Ni (ppm)	Sc (ppm)
Chrysotile							
Rhodesia (UICC A)	AERE [a]	1.7	1390	55	450	1360	6
" "	DHEW [b]	0.6	1378	54	393	1482	—
" (UICC AR)	DHEW	0.91	1170	43	231	1284	—
Canada (UICC) B	AERE	2.6	490	50	480	820	5
"	DHEW	1.24	317	45	444	802	—
Canada (UICC BR)	DHEW	1.08	317	46	419	873	—
Canada (Mine A)	AERE	4.8	515	63	540	720	5
" (Mine B)	AERE	3.5	780	110	580	1820	4
" (Mine C)	AERE	2.0	1200	60	420	1510	12
" (Mine D)	AERE	4.1	930	78	600	840	7
" (Mine E)	AERE	3.2	435	57	610	540	5
" (Mine F)	AERE	2.9	730	78	450	1790	8
" (Mine G)	AERE	1.9	480	44	420	1520	8
" (Mine H)	AERE	3.2	380	53	530	330	6
Cyprus	AERE	3.1	340	54	720	870	2
Amosite							
South Africa (UICC)	AERE	M [c]	35	7	11800	<100	5
" "	DHEW	M	31	11	13350	33	—
Crocidolite							
South Africa (UICC)	AERE	M	16	2	880	<100	0.5
" "	DHEW	M	20	10	833	8	—
NW Cape (Mine A)	AERE	M	<20	0.6	240	<100	<0.1
" " (Mine B)	AERE	M	<20	0.4	170	<100	0.3
Transvaal (Mine A)	AERE	M	20	0.8	140	<100	0.6
" (Mine B)	AERE	M	<20	0.6	220	<100	0.3
Anthophyllite							
Finland (UICC)	AERE	4.4	870	50	1060	1360	5
"	DHEW	2.0	584	24	986	414	—

[a] Atomic Energy Research Establishment, Harwell, UK.
[b] Department of Health Education and Welfare, Cincinnati, USA.
[c] Massive.

scandium by NAA, but the other elements can be determined with adequate sensitivity by either technique. NAA is more sensitive than AAS for evaluating the levels of the majority of elements, and this is an advantage when small samples are to be analysed. For example, Morgan & Timbrell (1971) used NAA to determine the amounts of chrysotile and crocidolite in 10 mg samples of blended asbestos and also to show that the UICC sample of Canadian chrysotile

Emission spectrography has also been used for the analysis of a wide range of elements in the UICC samples (Timbrell, 1970). The accuracy of this method is, however, inferior to that which can be achieved with either AAS or NAA.

As shown in Table 1, the levels of trace metals in all the chrysotile samples fall within well-defined ranges; thus it does not appear that the source of a sample can be identified by its trace element comple-

ment. With the possible exception of the manganese of Chrysotile A, it appears that the UICC standard reference samples were not significantly contaminated during the milling process which was required to produce a high proportion of respirable material from the rough-milled samples (AR and BR in Table 1).

In addition to making measurements of trace metals in bulk samples of asbestos, Cralley *et al.* (1968) also determined the same metals in < 10 μm fractions, prepared by the sieving method of Kupel *et al.* (1968). They showed that, in some cases, the composition of the < 10 μm fractions differed considerably from that of the bulk material. As this sieving process must have the effect of concentrating the non-fibrous material in the < 10 μm fraction, a change in composition relative to the bulk material would be expected. Gibbs (1971) measured levels of cobalt, iron, manganese and nickel in different grades of chrysotile fibre from a number of mines in Quebec and found that the shorter grades invariably contained the highest concentration of all four metals.

Solubility of chrysotile asbestos

The solubility of chrysotile in mineral acids has been studied by a number of workers. Acids attack chrysotile by reacting with the hydroxyl groups on the surface of the fibrils. Electron micrographs of partially decomposed fibrils show that they consist of an inner layer of intact chrysotile and an outer layer of silicaceous residue. As decomposition proceeds, the electron diffraction pattern becomes weaker, although the fibre morphology is retained. Atkinson & Rickards (1971) showed that at room temperature, the rate of attack is directly proportional to acid concentration provided this is greater than 1 N; at lower concentrations, however, the rate of attack decreased more slowly, possibly because the diffusion of magnesium becomes the rate-controlling process. The reaction rate with fibrillar chrysotile is independent of acid type, provided the acid is fully ionised. Morgan *et al.* (1971b) studied the rate at which magnesium was leached from chrysotile fibres in N hydrochloric acid at 25°C. Leaching curves were obtained which showed that milling increases the magnesium solubility, but that the origin of the fibre is also an important factor. Intrinsic differences in solubility are attributed mainly to variations in the porosity of fibre bundles, but fibril diameter and the nature of the interfibrillar material may also play a part.

Using neutron-activated chrysotile, the same workers compared the leaching curves of the associated chromium, cobalt, iron and scandium with that of the magnesium; they were thus able to determine the fractions of these elements which were present as isomorphous substitutes for the magnesium. Virtually all the scandium appears to be present in this form in all the samples examined, presumably because its ionic radius is close to that of magnesium (Whittaker & Muntus, 1970). Generally, most of the non-magnetic iron, chromium and cobalt could be accounted for in the octahedral (brucite) layer of the fibre. In some samples from Quebec, however, a considerable fraction of the chromium is present in an insoluble non-magnetic phase (probably chromite).

Cralley *et al.* (1968) showed that trace metals associated with asbestos have a finite solubility in bovine serum; and Holmes & Morgan (1967), using neutron-activated chrysotile, found that the associated chromium and cobalt dissolved at a significant rate after its administration to rats by intrapleural injection.

Evidence is accumulating that chrysotile magnesium dissolves fairly readily *in vivo*. Langer *et al.* (1970) used the electron microprobe to analyse the Mg:Si ratio in fibres isolated from the lungs of animals and of workers occupationally exposed to asbestos. In both series they found ratios consistent with magnesium-depleted chrysotile. Recently, Pooley (1972) has examined chrysotile fibres, isolated from human lung, by electron diffraction and has been able to obtain characteristic diffraction patterns. Work with magnesium-depleted chrysotile, obtained by acid leaching *in vitro*, showed that such patterns could be obtained from fibres with up to half their magnesium removed; the fibres isolated from lung could therefore have lost up to half their magnesium *in vivo*.

Morgan *et al.* (1971a) have used a radioactive tracer technique to obtain information on the rate of magnesium dissolution *in vivo*. They selected samples of chrysotile in which most of the cobalt is present in the octahedral layer as an isomorphous substitute for magnesium and, after neutron irradiation, injected them intrapleurally into rats. As leached cobalt is excreted quite rapidly, the excretion of ^{60}Co was used as an index of magnesium solubility. The results indicate that the magnesium on the surface of the fibre (which accounts for 5–10% of the total) dissolves within a few days of administra-

tion. After 2 months, about 30% of the magnesium had dissolved; but after this period, further leaching occurred only very slowly.

AMPHIBOLE ASBESTOS

Composition

Idealised chemical formulae for the three main types of amphibole asbestos are given below. Where cations are written in parentheses without subscripts a variable composition is indicated, with the most abundant species first.

Crocidolite	$Na_2Fe_3^{+}{}^{+}Fe_2^{+}{}^{+}{}^{+}Si_8O_{22}(OH)_2$	
Amosite	$(Fe^{+}{}^{+}Mg)_7$	$Si_8O_{22}(OH)_2$
Anthophyllite	$(MgFe^{+}{}^{+})_7$	$Si_8O_{22}(OH)_2$

The variability in composition is due to the fact that the crystal structure can accommodate many ions in the spaces between the silica ribbons, and it is the variable nature of host rocks which contributes different ions to this structure.

Associated trace elements

As shown in Table 1 the levels of chromium, nickel and cobalt in amosite and crocidolite are generally one or two orders of magnitude lower than in chrysotile. The levels of these elements in anthophyllite are, however, very similar to those in chrysotile. Manganese is found in rather similar concentrations in all the UICC samples, with the exception of the amosite which contains 1.4% of this element.

Solubility of amphiboles

The amphiboles are much more resistant to attack by acids than is chrysotile. From unpublished work referred to by Hodgson (1965) it appears that the weight loss in refluxing 4N hydrochloric acid is in the order:

amosite $\gg$ crocidolite $>$ anthophyllite.

More recent leaching studies (Morgan *et al.*[1]) with the UICC reference samples show that in 0.1 N hydrochloric acid at 25°C the structural iron of amosite is slightly more soluble than is that of crocidolite, 9 and 7%, respectively, being dissolved in 28 days. In neutral phosphate/citrate buffer at the same temperature, about 3% of the iron dissolved in the same period. The iron of the anthophyllite was considerably more soluble than was the magnesium, which indicates that these elements may be located at different sites in the crystal structure of this amphibole.

Excretion of ^{59}Fe by rats which had been injected intrapleurally with neutron-irradiated amosite and crocidolite (Holmes & Morgan, 1968) showed that the structural iron dissolves *in vivo*, but at a much slower rate than does the magnesium of chrysotile. Langer *et al.* (1972) measured the Mg:Si ratios in amphibole fibres isolated from lungs of asbestos workers and compared them with the corresponding ratio in the appropriate UICC reference sample. The magnesium loss from amphiboles in the lung appeared to follow the sequence:

anthophyllite $>$ amosite $>$ crocidolite.

DISCUSSION

Harington & Roe (1965) suggested that asbestos carcinogenesis could be due to the presence of trace metals such as chromium or nickel, which are known to be carcinogenic under certain circumstances. An alternative hypothesis has been put forward by Dixon *et al.* (1970), who suggested that trace metals associated with asbestos inhibit the metabolism of benzpyrene, thus increasing the residence time of this carcinogen in the lung. They found that nickel, chromium and beryllium were the most effective inhibitors of benzpyrene hydroxylase. If this theory is correct, then the role of asbestos is purely that of a passive carrier of trace metals. Recently, Cralley (1971) has indicated that alloy metal particulates associated with asbestos fibres could give rise to high concentrations of biologically active cations at localised tissue sites, and that these could be responsible for asbestos carcinogenesis. Metals such as manganese, which are high in the electromotive series, could have the effect of precipitating metals lower in the series when they dissolve.

If the role of trace metals in asbestos carcinogenesis is to be elucidated, more information is required on their distribution between fibre and contaminating minerals and alloys. Attempts to correlate carcinogenicity with trace metal concentration *per se* are pointless, since it is clear that they can be present

[1] Unpublished data.

in a variety of chemical and physical forms with varying solubility. As evidence accumulates that chrysotile magnesium dissolves at a finite rate *in vivo*, the effect of magnesium removal on the cytotoxicity and carcinogenicity of chrysotile should be investigated.

SUMMARY

The chemical characteristics of asbestiform minerals are described, with particular reference to the UICC standard reference samples. Measurements of the levels of chromium, cobalt, iron, manganese, nickel and scandium in a range of samples are reported, together with information on their distribution between fibre and accessory minerals. The solubility of structural and trace constituents of asbestos, both *in vitro* and *in vivo*, is discussed.

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Identification of single asbestos fibres in human tissues

A. M. LANGER [1] & F. D. POOLEY [2]

THE ASBESTOS MINERALS

The asbestos mineral group consists of five fibrous, hydrated silicates, which have a number of uniquely useful physical and chemical properties rendering them essential to our industrial society (Rosato, 1959). The commercially-used asbestos minerals include amosite, anthophyllite, chrysotile, crocidolite and tremolite (Speil & Leineweber, 1969). These mineral fibres are representative of complex chemical systems; for example, crocidolite is constituted of molecular proportions of the minerals glaucophane,

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TABLE 1. PROPERTIES OF THE ASBESTOS MINERAL FIBRES USEFUL FOR IDENTIFICATION

Amosite
Gross Morphology: Chain silicate; fibrous cleavage.
Mineral Group: Amphibole.
Chemical Composition: $Fe_7Si_8O_{22}(OH)_2$. Some Mg, Fe > 5.
Refractive Indices: Nx = 1.678, Nz = 1.695.
Morphology [a]*:* Tends to range in thickness from 0.1–0.9 μm with a maximum between 0.25–0.50; fibres tend to be electron dense and straight; some diffraction contrast figures on thinner fibres and at high voltages. Diffraction pattern of amphibole group.

Anthophyllite
Gross Morphology: See amosite.
Mineral Group: See amosite.
Chemical Composition: $Mg_7Si_8O_{22}(OH)_2$. Some Fe, Mg > 6.
Refractive Indices: Nx=1.596–1.654, Nz=1.625–1.666.
Morphology [a]*:* Tends to form fibres thicker than amosite; generally electron dense and straight. Diffraction pattern of amphibole.

Chrysotile
Gross Morphology: Sheet silicate rolled about a central capillary; fibres are bundles of fibrils.
Mineral Group: Fibrous member of the serpentine group.
Chemical Composition: $Mg_6Si_4O_{10}(OH)_8$. Some Fe, Mg > 5.
Refractive Indices: Nx = 1.516–1.549, Nz = 1.524–1.556.
Morphology [a]*:* Both unit fibril and fibril bundles are common; internal capillary surrounded by an electron-dense wall is common for fibril; fibre bundles consist of fibrils; fibres tend to be broken open at the end and "shredded"; clumps are common; fibres are commonly curved; some beam-damaged fibrils are invariably present. Electron diffraction pattern is unique for chrysotile.

Crocidolite
Gross Morphology: See amosite.
Mineral Group: See amosite.
Chemical Composition: $Na_2Fe_2^{++}Fe_3^{+}Si_8O_{22}(OH)_2$. Some Mg, some Ca.
Refractive Indices: Nx=1.655–1.701, Nz=1.668–1.717.
Morphology [a]*:* Forms fibres thinner than amosite; fibres tend to be electron translucent with diffraction contrast figures commonly observed along the fibre length; fibres tend to be curvilinear. Diffraction pattern of amphibole.

Tremolite
Gross Morphology: See amosite.
Mineral Group: See amosite.
Chemical Composition: $Ca_2Mg_5Si_8O_{22}(OH_2)$. Some Fe, Mg > 4.
Refractive Indices: Nx = 1.581–1.615, Nz = 1.601–1.641.
Morphology [a]*:* Thick, stubby, fibres are commonly observed; diffraction contrast figures are rarely seen on these fibres; fibres are always "straight". Diffraction pattern of amphibole.

[a] Seen under the transmission electron microscope.

riebeckite and crossite, depending on its sodium, iron, magnesium and aluminium contents (Ernst, 1968). Thus the asbestos minerals themselves are only the representative physical-chemical entities of more complex silicate systems. The common asbestos minerals are listed in Table 1, with those of their properties which are useful for single fibre identification.

EXAMINATION OF TISSUES FOR FIBRES

The amount of tissue to be examined for asbestos fibres is largely determined by the intensity of exposure (occupationally exposed *versus* non-exposed) of the subjects, and on the material available for study. If tissue is available from individuals with intensive fibre exposure, little material is required in order to localise fibres; histologic sections are adequate in these cases. In instances in which little asbestos is likely to be encountered, the use of bulk tissue will significantly increase fibre yield and greatly reduce the time necessary for fibre search. Histologic sections are generally inadequate, since localisation of fibres during a relatively short search is highly dependent on chance.

The technique used for the recovery of inorganic fibres from bulk tissue was the KOH digestion method, as outlined by Langer & Selikoff (1971). Essentially; dried lung tissue is digested in a 5% KOH solution at 90°C for 4 hours. Several cycles of washing and centrifugation of the residue are required for the complete removal of digested materials and of the KOH. The residue is then dispersed in triple-distilled water by sonification, and a small amount is pipetted onto an appropriate electron microscope substrate. The major advantage in using this method is that particles are concentrated from a much greater amount of tissue than one would normally encounter on a histologic slide. The major disadvantage is that the size-distribution pattern observed in such preparations is often an artefact. Chrysotile fibres, for example, are readily reduced into fibril form.

Histologic sections for fibre analysis may be prepared by a number of methods. The technique to be selected depends on the type of instrumentation which will be used for fibre localisation and identification. The carbon-extraction technique (Pooley, 1972; Langer *et al.*, 1972b) is now used routinely in our laboratory in the preparation of tissues for examination with electron beam instruments. The preparation involves the ashing of a 5–8 μm thick, unstained, histologic section mounted on a glass slide; the relict tissue is then impregnated in a water-soluble plastic (polyvinyl alcohol). Then the hardened plastic and its incorporated ashed tissue are "peeled" from the slide; the relict tissue is then inverted, and a thick carbon coat is deposited onto the inverted surface. The plastic is dissolved from the carbon sheet (which now includes the relict tissue and the inorganic particles); and finally, the carbon film is placed onto appropriate substrates for examination in an electron beam instrument (EM copper locator grids are best for this purpose).

INSTRUMENTATION FOR IDENTIFICATION OF FIBRES

The instruments available for the identification of single particles in lung tissue include the light microscope, the electron microscope (those capable of routine changes in "column geometry" for obtaining electron diffraction images), and the electron microprobe analyser. The recommended instrument characteristics, the information obtained from each, their limitations, and their direct application to the asbestos fibre problem, are given in Table 2. It is apparent from the information given in this table that the light microscope is of limited use for the identification of fibres in human tissues; many difficulties are inherent in this method, not the least of which is the severe size limitation. The electron beam instruments have been demonstrated to yield sufficient morphological, structural and chemical data to uniquely identify the common asbestos fibre types.

IDENTIFICATION OF ASBESTOS FIBRES WITH ELECTRON BEAM INSTRUMENTS

Chrysotile

The physical and chemical properties of chrysotile asbestos are such that many of the fibres observed in human tissues are below the resolution of the light microscope (Langer *et al.*, 1971; Pooley *et al.*, 1970; Pooley, 1972). The search for such fibres is easily accomplished with a transmission electron microscope in which individual unit fibrils can easily be resolved. The fibril morphology is so unique that the mineral species may be identified on this basis

alone (Langer *et al.*, 1971; Langer *et al.*, 1972a). It is characterised by an internal central capillary surrounded by electron-dense walls, which is often encapsulated in an electron translucent "amorphous" material (Fig. 1). Chrysotile fibres are made up of bundles of fibrils, which are often broken open (Fig. 2; and see figures in Pooley, 1972). It is not unusual to observe fibrils, fibres, and odd-shaped "clumps" of chrysotile in the lung tissues of individuals who have been occupationally exposed to

TABLE 2. CURRENTLY AVAILABLE INSTRUMENTS WHICH MAY BE USED FOR SINGLE ASBESTOS FIBRE IDENTIFICATION

Light microscope

Recommended instrument: Polarising light optics; range of magnifications up to 500×; to be used with immersion oils.

Information obtained: Particle morphology and shape, including length:width ratios; fibre size; colour in plane polarised light; pleochroic properties; birefringence and crystallinity; refractive indices; extinction properties.

Limitations: Size restrictions (Abbe's Law), only the largest fibres may be seen; optical properties change with decreasing particle size; minerals alter *in vivo*; organic and inorganic body materials coat fibres, altering their optical properties; localisation in tissues is difficult, and more so with decreasing particle size.

Asbestos fibres: Most of the chrysotile content of the human lung will not be seen by light microscopy; amphibole asbestos minerals tend to overlap; well-defined exposures are required for identification of fibre types. The light microscopic method of fibre identification is extremely limited.

Electron microscope

Recommended instrument: Transmission electron optics; range of magnifications up to 100,000× directly onto the screen; darkfield microscopy; selected area diffraction with range of camera lengths; accelerating voltages up to 125 kV; goniometer stage.

Information obtained: Particle morphology and shape, including length:width ratios; no small size restriction for fibres; differing electron densities for fibre types; differing diffraction contrast patterns for different fibre types; selected area diffraction patterns obtainable (single crystal geometry with Laue characteristics).

Limitations: Upper size limit, in that large fibres are generally excluded from analysis; amphibole fibres, occurring as single particles, are difficult to differentiate morphologically; amphibole diffraction patterns tend to overlap.

Asbestos fibres: Chrysotile may be uniquely characterised by its morphology or its electron diffraction pattern; groups of amphibole fibres (single fibre type) in a well-defined exposure may be identified morphologically; single amphibole fibres or fibres in a mixed exposure are less well identified; selected area diffraction patterns tend to overlap and are often indistinguishable; mineral group may be identified (amphibole) but not individual species. The electron microscope is recommended as one of the basic instruments for fibre identification.

Electron microprobe

Recommended instrument: Dispersive crystal analysers; beam control for both raster sweep and point mode operation; scanning mode and transmission mode of particle localisation; magnifications up to 10,000×; optimum resolution in the scanning EM range; up to 30 kV beam acceleration; range of analysing crystals for light-element range (to boron).

Information obtained: Morphology; length:width ratios; sizes of fibres greater than 200 nm; chemical analyses on total particle or portion of particle; qualitative or quantitative chemical analyses.

Limitations: Most electron probes have poor resolutions in the scanning mode of operation, to about 0.2 μm, thereby excluding many asbestos fibres from analysis; localisation of particles is difficult and time-consuming; detailed fibre analysis requires localisation of fibres first with a transmission electron microscope then transference of grids into the probe for analysis; micromanipulation techniques are totally inadequate in that they entail a population selection; detection of chemical elements is now restricted to those in the periodic table higher than boron; length of time for analysis is related to the number of elements sought.

Asbestos fibres: Chrysotile analysis is restricted to bundles rather than thin fibres; fibrils are excluded from analysis; chrysotile is degraded (leached) of magnesium *in vivo* so that characteristic chemical analyses are rarely obtained; silicon-rich fibres may be chrysotile "ghosts" or other materials (these data are considered equivocal); amphibole asbestos fibres are readily distinguishable from each other on a microchemical basis; each commercial fibre type possesses a narrow range of bulk chemical characteristics in the Ca–Na–Fe–Si–Mg system; individual amphibole asbestos fibres may be uniquely identified on a chemical basis in several minutes of analytical time. The probe should be considered as a secondary instrument to be used in conjunction with a transmission electron microscope. It is essential for amphibole analysis and may be useful in chrysotile analysis.

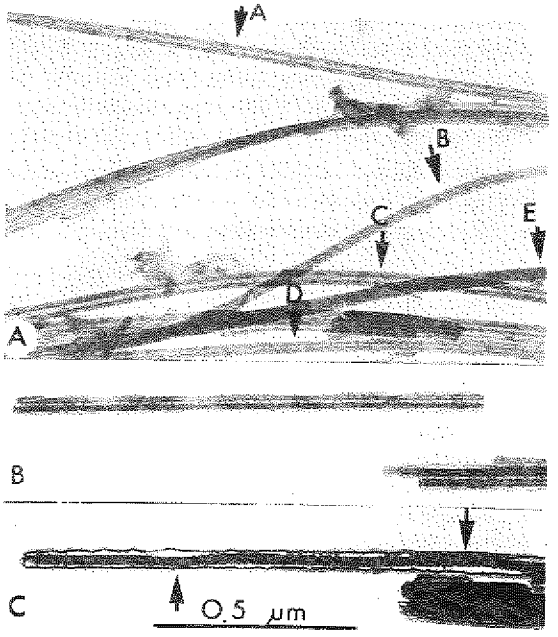

Fig. 1. Micrographs of chrysotile. Scale as marked.

Transmission electron micrograph of chrysotile fibrils.

(A) Fibrils showing a range of capillary and wall dimensions: *A* 38.5 nm fibril with 10 nm capillary; *B* 36 nm, with a 4 nm capillary; *C* 45 nm, with varying wall and capillary; *D* 38 nm, with a 6 nm capillary; *E* 44 nm, with no central capillary.

(B) Fibril showing incipient beam damage, with the development of an electron translucent layer of dehydroxylated material on its surface.

(C) Fibril displaying more extensive beam damage. A thick layer of dehydroxylated material encapsulates the entire fibril. The internal crystalline wall is deformed within this envelope, and appears "bent" (see arrows).

Single fibrils in tissues may have any or all of these characteristics.

this form of asbestos. In addition to its unique morphology, chrysotile also possesses a characteristic electron diffraction pattern (Figure 3[A]; and see Zvyagin, 1967), thus, its presence in a tissue can be established with little problem (Langer *et al.*, 1971; Pooley, 1972).

Attempts have also been made to identify chrysotile fibres in tissues chemically, utilising the electron microprobe analyser (Langer *et al.*, 1970; Langer *et al.*, 1972a, b). These attempts have met with limited success, since *in vivo* chrysotile tends to degrade both chemically and physically (Suzuki & Churg, 1969; Morgan & Holmes, 1970); the fibres tend to break down into units of a size not easily resolved by the probe and to lose magnesium readily.

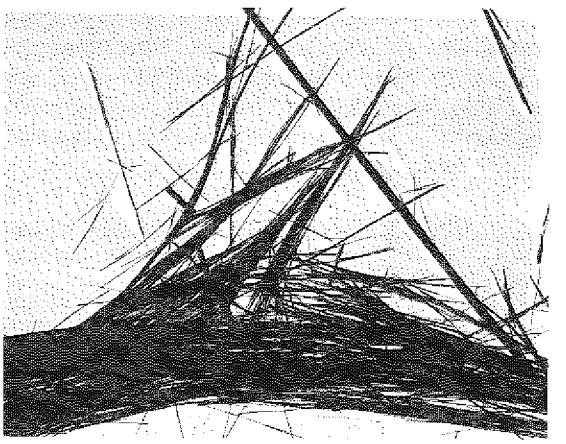

Fig. 2. Micrograph of chrysotile.

Transmission electron micrograph of a chrysotile fibre breaking open into its unit fibrils. High magnification examination of the units shows the capillary structure. The fibrils tend to display marked curvature when breaking off the fibre bundle. Electron diffraction on such a bundle displays a characteristic chrysotile pattern. Magnification is about 3000× (fibre is about 5μm thick).

Amphiboles

This asbestos group includes the common minerals amosite, anthophyllite, crocidolite and tremolite. When large fibres are visible in tissues, light microscopy may be employed to define, in part, the asbestos type. However, the optical properties of these fibres may be ambiguous, since they may have altered *in vivo* or may be obscured by coatings formed

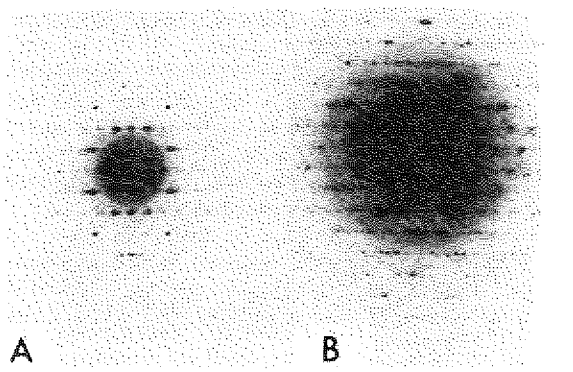

Fig. 3. Asbestos diffraction patterns.

Electron diffraction patterns for chrysotile (A) and amosite (a representative amphibole) (B). The horizontal arrays represent similar spacing elements in their structures (*c*-axis amphiboles, 0.53 nm; *a*-axis chrysotile, 0.53 nm). Both axes are the respective fibre axes of the minerals. The closely spaced points in the amphibole pattern indicate the large d-spacings.

through body reactions ("asbestos bodies"). Also, many of the fibres may be smaller than the resolving power of the light microscope, giving a faulty impression of the total number of particles present and of their distribution. The optical properties of the fibres in such small size ranges are non-distinctive and cannot be used for absolute identification. An electron beam instrument is necessary for the identification and characterisation of amphibole asbestos fibres in tissues.

Electron microscopic examination of amphibole fibres shows them to be electron dense and generally straight (Fig. 4). The different fibre types tend to

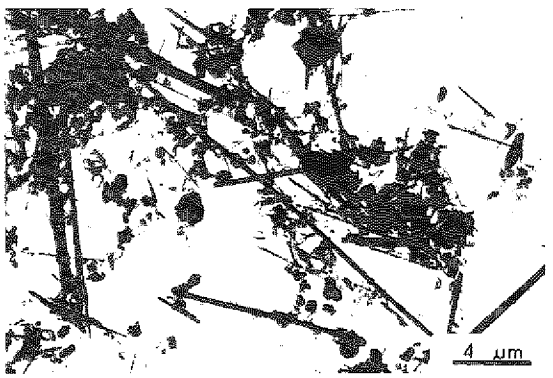

Fig. 4. Micrograph of amosite.

Transmission electron micrograph of amosite fibres. These fibres show most, if not all, of the common features of the amphibole asbestos group. Fibres tend to be electron-dense and straight. Some fibres display a cross-banding of alternating light and dark areas which are produced by electron diffraction contrast. The electron diffraction pattern obtained on one of the fibres in the field is shown in Fig. 3(B).

range characteristically in thickness, electron translucency and diffraction contrast patterns (Table 2). However, single fibres tend to be remarkably similar and cannot easily be differentiated. It has been shown that when many amphibole fibres are present in tissue, the fibre type may be identified with reasonable certainty, if the exposure has not been "mixed" (Timbrell *et al.*, 1970). Each of the fibre types appears to possess a unique diameter distribution: anthophyllite fibres are significantly thicker than amosite fibres, which in turn are significantly thicker than those of crocidolite; tremolite fibres tend to be short and stubby. Unfortunately, most asbestos exposures are mixed, so that the value of morpho-

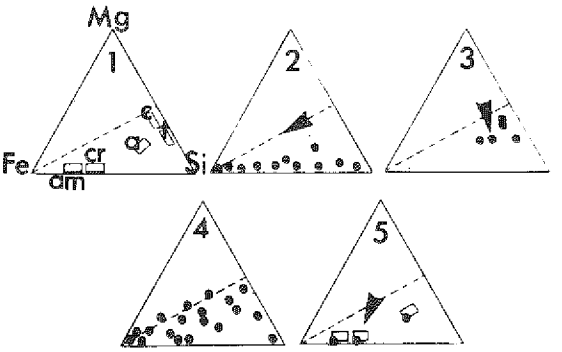

Fig. 5. Probe analyses.

Schematic emission diagrams of asbestos fibres and bodies analysed in the electron microprobe. All diagrams, 1–5, represent completed analyses.

(1) Analyses obtained on standard fibres obtained from many sources; each fibre type defines a well-delineated field in the Fe–Si–Mg X-ray system; each fibre type may be differentiated on this basis: *c* = chrysotile, *t* = tremolite, *a* = anthophyllite, *am* = amosite, *cr* = crocidolite.

(2) 50 analyses of asbestos bodies and their fibrous cores from individuals in the general population of New York City. The analyses are consistent with those of magnesium-depleted chrysotile fibres coated with ferritin. The arrow indicates the major trend of analyses.

(3) 12 analyses of asbestos bodies extracted from the lung of a Canadian asbestos worker exposed to chrysotile. The major trend defined by the analyses is that of magnesium-leached fibres. The ferritin coating inclined the analyses towards the iron apex (arrow).

(4) Analyses of 20 asbestos bodies extracted from the lung of a hamster. The range of the analyses indicates that the cores have variable amounts of magnesium remaining in the fibre. Some fibrous cores have been totally leached of magnesium. The animal was exposed to chrysotile asbestos.

(5) Analyses of 77 uncoated fibres observed in the lung tissues of workmen with known and well-defined fibre exposure. The standard fibre fields are marked (boxes), as are the obtained fibre analyses (circles). The arrows show that each of the trends indicate a magnesium loss from the amphibole fibres.

logical analysis for fibre identification is limited. In addition, electron diffraction patterns obtained on amphiboles are also non-distinctive (Fig. 3[B]); the pattern is unique for the mineral group, but not for the individual mineral species. Instrumental variations, errors in pattern measurement, structural defects and twinning produce overlapping patterns. The surest means of differentiating among the amphibole fibres is by microchemical methods.

Fibres which have been examined with the transmission electron microscope may be examined with an electron microprobe analyser merely by transferring the grid into the latter instrument.[1] With this instrument the amphibole asbestos types have

[1] The probe and the transmission electron microscope are described here as two separate instruments. There are instruments, e.g., the AEI EMMA (electron microscope microprobe analyser), which incorporate the two into a single unit.

TABLE 3. RECOMMENDED ANALYTICAL PROCEDURE FOR SINGLE FIBRE IDENTIFICATION

Tissue preparation: Use of the carbon extraction technique is recommended since it can be used on normal histologic preparations and the carbon substrate is stable under the electron beam; the grids should be mounted on locator-type copper substrates to ensure ease of particle location.

Light microscopy: This phase of examination should be used only to check on the extraction preparation and the general condition of the grids; areas which contain visible fibres and amounts of relict tissue should be selected; areas which appear to be void of fibres should be scanned as well.

Electron microscopy and selected area diffraction: Photographs of fibres should be taken as a basis for their morphological identification; selected area diffraction patterns are necessary on all electron-dense fibres to determine if they are amphibole types; the locations of these fibres should be noted for further microchemical analysis with the probe.

Electron microprobe: Fibres on which diffraction and morphological studies have been completed can be located by means of the locator grids and microchemical analyses obtained.

been differentiated on a microchemical basis (Langer *et al.*, 1970; Langer *et al.*, 1972a, b; and see Fig. 5). The probe has been used to characterise the fibrous cores of asbestos bodies as well as uncoated fibres, in both human and animal tissues. It is at present the most reliable method for the identification of amphibole fibres. There are some drawbacks in using the probe as a standard analytical tool (Table 2); the most important of these involve the time of scan for particle location, and the size limitation imposed; i.e., only the largest fibres (greater than 0.2 μm in thickness) are analysed without difficulty and with high confidence.

RECOMMENDED ANALYTICAL PROCEDURE

The identification of single asbestos fibres in tissues may be accomplished by the use of electron beam instruments. These instruments include the transmission electron microscope and the electron microprobe analyser. Morphological, structural and chemical data complement each other for unique identification of the fibre types. The tissue preparation may involve either bulk digestion of gross specimens or the use of normal histologic sections prepared by the carbon extraction technique. The analytical procedure is outlined in Table 3.

SUMMARY

Preparative techniques have been developed and instruments are available which permit the localisation, characterisation and identification of single asbestos fibres in human tissues. Utilising the carbon extraction technique standard histologic sections may be prepared, and can be examined with the transmission electron microscope and the electron microprobe analyser.

Remnants of ashed tissues, in which gross histologic features and their inorganic particulate burden are preserved, may be impregnated into a stable carbon matrix. This preparation permits prolonged examination of particles under an electron beam without the formation of artefact and with no particle loss. By means of transmission electron microscopy, chrysotile asbestos may be readily differentiated from all other asbestos fibre types. Its unique morphology, consisting of an electron-translucent capillary surrounded by a more dense crystalline "wall", coupled with its characteristic electron diffraction pattern, uniquely identify this fibre type.

Generally, the amphibole asbestos minerals have a morphological resemblance to each other and are not readily distinguishable on an individual basis. This is especially true in cases where the number of fibres is small and a statistical morphological analysis of their width distribution is not satisfactory; and in cases of mixed fibre exposure, the characteristic width distribution patterns are equivocal. Selected area diffraction patterns are ambiguous as well, since they indicate only the mineral group and not the individual mineral species. In these latter cases, when amphibole asbestos fibres are present, the grids may be transferred into an electron microprobe analyser and subjected to microchemical analysis; on this basis, the amphibole asbestos minerals may be readily distinguished from each other.

All asbestos fibre types may be differentiated and identified on the basis of morphological, structural and chemical data.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the following support: AML under Career Award NIEHS ES 44812, and FDP support under a grant from the Medical Research Council and the PRU, Penarth.

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Asbestos in the environment

W. J. NICHOLSON [1] & F. L. PUNDSACK [2]

This paper will discuss the sources from which asbestos may enter the air and water of our environment. Methods of measuring small quantities of asbestos will be described, and data on the quantities which have been found in the atmosphere and in rivers will be presented. Throughout the discussion, the term "asbestos" will be used to refer in a non-specific way to the family of fibrous inorganic minerals typified by chrysotile, crocidolite, amosite, anthophyllite, actinolite and tremolite.

SOURCES

There are two general conditions under which asbestos fibres may be freed in a form to enter our environment: (*a*) the activities of man; and (*b*) the activities of nature. Man's activities encompass the mining and milling of asbestos, or of other materials that contain asbestos; the fabrication of asbestos-containing products; the application and use of these products; and the disposal of the waste or residue from these activities. The natural source of asbestos in our environment is from the weathering and erosion by wind and water of rock masses that contain asbestos.

The mining of asbestos is carried out in both open-pit and underground mining operations. The potential sources of asbestos in the environment are open blasting of ore, transportation of the ore to the milling site, drying the ore, freeing the fibre from the host rock, and disposal of the waste or tailings from the milling process. In addition to the mining of asbestos ores, other mining operations, which involve materials that may contain asbestos as a minor impurity, are potential sources of asbestos. Examples would be the mining of certain talc deposits which contain tremolite asbestos as an impurity, and the quarrying of serpentine rock which may contain asbestos. Although quarry grade serpentine rock may not contain any visible asbestos fibres, examination with the electron microscope of a number of serpentine rock samples from many different geographical sources has shown that in many of them these fibres do occur.

Manufacturing operations which incorporate asbestos into the many products in which it is used are potential sources of asbestos in the environment unless all air and water effluents from the manufacturing activities are efficiently and effectively filtered. In addition, the solid asbestos-containing waste from manufacturing is a source of contaminating asbestos unless it is properly transported to a suitable waste-disposal area and well covered.

The major world-wide use of asbestos in manufacturing is in combination with cement to form a variety of asbestos-cement products, such as pipes, flat and corrugated sheets, shingles and moulded articles. Well over half of all the asbestos produced annually is incorporated into asbestos-cement products of this type. Other significant uses of asbestos are in asphalt and vinyl-based flooring, in insulating products for pipes and boilers and shipboard walls, in roofing felts and shingles, in special textiles, in friction materials and packings, in gypsum joint cements, in spray composition fireproofing products and in electrical insulations. There are literally hundreds of applications beyond those just listed, but many of them constitute relatively minor uses in so far as the total annual consumption of asbestos is concerned.

Once an asbestos-containing product has been manufactured, whether or not it constitutes a source of asbestos in the environment will depend to a great

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extent on whether or not the asbestos is firmly "locked-in" the product with a binder, saturant, coating or bonding agent such that normal handling, application and use do not release it. Asbestos-cement products are a good example of "locked-in" products which probably do not constitute a significant source of asbestos to the environment under normal conditions of use. On the other hand, the use of asbestos in spray-on fireproofing compounds is an example of a non-locked-in use. The major source of environmental contamination in this case is the application of the spray itself and the subsequent dissemination of airborne asbestos fibre from the application site. This problem is serious enough that asbestos spraying has been banned in a number of cities in the United States.

Another potential source of asbestos in the environment may be brake-linings, which contain about 50% asbestos by weight. Studies have shown that under average braking conditions 98% or more of the asbestos in the linings is destroyed by the heat of friction. Nevertheless, it has been speculated that the small quantities of asbestos which are released from the linings may contribute to the background level of electron-microscope-size fibres in the ambient air. More definitive work needs to be done to establish the actual quantity of asbestos fibre released from brake linings in a state which permits it to become and remain airborne.

The demolition of structures which contain asbestos-bearing products can be a source of fibre in the air, unless precautions are taken to wet down the asbestos-containing products. This is particularly true for the demolition of relatively low-density products such as pipe and boiler insulation and of buildings which had been fireproofed with an asbestos-containing spray.

Construction excavations and road building activities can be potential sources of asbestos in the environment, if they take place in areas of serpentine rock. Since most serpentine rock contains electron microscopic asbestos fibres, the open blasting associated with road building and excavations could release such fibres into the air.

The natural weathering of asbestos-bearing serpentine rock is another potential source of electron microscopic fibre in both the ambient air and in water. Serpentine is a relatively common type of rock in many areas of the world, where it occurs at or near the surface. Although it is reasonable to assume that the weathering of these rock masses may release some fibre to the environment, it is difficult to determine how much this source may contribute to the environmental background of asbestos.

SAMPLING AND ANALYSIS TECHNIQUES

In the analysis of ambient air samples for asbestos, the presence of other organic and inorganic material presents significant problems. Typical urban air may contain 100 $\mu g/m^3$ of "suspended particulates". Such material is generally of respirable size and may include 25 to 50% of inorganic matter. In contrast, typical urban asbestos concentrations range from about 0.1 ng/m^3 to perhaps 100 ng/m^3. Thus, asbestos may constitute only 0.0001 to 0.1% of the particulate matter present in a given air sample. Moreover, the asbestos found in the ambient air includes both micron-size fibres and numerous individual fibrils having diameters of from 20 to 40 nm and lengths of perhaps 100 nm. In many cases these fibres and fibrils may be agglomerated with a variety of other material present in the air sample.

These considerations preclude the possibility of quantitative analysis of such ambient air samples by light microscopy, bulk spectroscopic techniques or X-ray diffraction. The agglomeration of the asbestos with other materials and the presence of many sub-light microscopic fibres render light microscopy ineffective. Moreover, the unique identification of small optically visible fibres is not always possible, even using a petrographic microscope. Any bulk analysis method attempted to date has failed because of the presence of the much greater quantity of other inorganic and mineral material.

The only effective analysis method has required the use of the electron microscope. Samples for such analysis have been collected on millipore filters, usually with a nominal pore size of 0.8 μm and, in some cases, backed by a nylon mesh. While the effective pore size of these filters is larger than the diameter of many of the asbestos fibres of interest, it has been shown that the surface charge properties of the filter and of the asbestos fibres, as well as the circuitous path through the filter, allow virtually complete collection of all asbestos material.

In most circumstances, high volume pumps were used for the collection of samples, although battery-operated personal monitoring pumps also proved useful. Here, however, the low quantity of collected material increased the statistical variability of

the result and emphasised the effect of any background contamination introduced during the analysis procedure. An optimum sample has been found to be one in which about 0.5 m^3 of air was filtered through 1 cm^2 of filter.

To prepare a sample for analysis in the electron microscope, a portion of the sample, mounted on a microscope slide, was ashed in a low temperature-activated oxygen asher for 20 minutes to 1 hour. This served to remove the membrane filter material, all organic material in the collected sample, soot and other carbonaceous material. The residue, consisting mostly of fly ash and mineral matter, was dispersed on the microscope slide by grinding the sample with a watch glass in a solution of 1% nitrocellulose in amyl acetate for 5 to 10 minutes. The sample was dispersed fairly uniformly over two microscope slides by placing another glass microscope slide over the ground sample, and drawing the microscope slides apart. Upon evaporation of the amyl acetate, the dispersal was scanned for uniformity by light microscopy, and representative areas were chosen for transfer to electron microscope grids for scanning.

Alternatively, techniques have been used in which the sample was dispersed by ultrasonic energy while suspended in a detergent solution (Aerosol OT). By thus breaking up and dispersing the mineral matter, the asbestos fibres which agglomerate on other particles are made visible. However, this procedure destroys any information concerning fibre size distribution; it is possible only to determine the mass of asbestos in a sample.

In the former, "rub-out", procedure, all the material from an ashed sample (usually that on 1–3 cm^2 of the filter) is dispersed over a prescribed area of a microscope slide. If no losses occur during the ashing and rub-out, the amount of fibre in the sample can be determined by multiplying the amount of fibre measured on the area observed in the electron microscope by the ratio of this area to the total area of sample on both microscope slides. In practice, losses occur in both the ashing procedure and rub-out. These have been determined by periodic checks with doped samples and found to be in the range of 40 to 70%. In addition to the problem of using an "average loss correction", there is an unknown error introduced by the non-uniformity of the material deposited over the total slide area.

An alternative procedure is to ash a larger portion, or all, of the sample in a crucible and to disperse an aliquot during the rub-out. Here, it has been found useful to mix some radioactive Au^{198} with the ashed residue. The fraction of the initial sample which has been dispersed on an electron microscope grid can then be determined from the relative count rates of the grid and of the full sample.

The samples thus prepared were scanned either directly or on photomicrographs at magnifications of approximately $\times 40{,}000$. Identification of chrysotile was possible because of its unique tubular structure, and this was the only asbestiform mineral quantitated in our analysis. Typically, four to eight 100 μm squares of separate grids from each sample were scanned, and the mass of chrysotile fibres was determined by sizing each individual fibre. The fibre observed per square ranged from a few fibrils to over 100. Background contamination levels averaged less than two fibrils per square. This level was maintained, however, only by adherence to strict clean room procedures, as normal room air provided a ready source of contamination. While random statistical variations occurred in the number of fibres counted per square, greater variations arose from the random presence of groups of fibrils from large fibres that were incompletely dispersed during the rub-out.

During the course of this work, sixteen samples were analysed in duplicate. The samples were mostly those for which an unusually high or low value had been found on initial analysis, so that differences in replicate analyses would reflect variations seen both in samples having only a few fibres present on a grid square and in those with large fibres that may have been improperly dispersed. The average per cent deviation from the mean of all samples analysed in duplicate was 43%.

In view of these calibration studies and duplicate analyses, we feel confident that a single analysis is accurate within a factor of two. Averaging over several samples thus provides a very good representation of the chrysotile asbestos content of urban air.

RESULTS OF SAMPLING AND ANALYSIS OF AIR

One hundred and eighty-seven (187) samples from 49 United States cities, collected by the National Air Pollution Control Administration by their air sampling network during 1969 and 1970, were analysed. Bi-weekly samples in various 3-month periods were composited and analysed, using the microscope slide

Table 1. Chrysotile content of ambient air—samples collected by NAPCA

Fibre range in 10^{-9} g/m³	No. of samples in range
0.1– 0.9	61
1.0– 4.9	102
5.0– 9.9	12
10.0–19	9
20 –49	2
50 +	1
Total samples	187

ashing procedure. Table 1 gives the ranges of values obtained. Table 2 lists the results obtained from a series of single samples collected in New York City by the Department of Air Resources at 12 sites of their sampling network.

Table 2. Chrysotile content of ambient air in New York City by borough

Sampling locations	Number of samples	Asbestos air level in 10^{-9} g/m³	
		Range	Average
Manhattan	7	8–65	30
Brooklyn	3	6–39	19
Bronx	4	2–25	12
Queens	4	3–18	9
Staten Island	4	5–14	8

All of the above samples were collected in periods during which the procedure of fireproofing high-rise buildings by spraying asbestos-containing materials was permitted. While no sampling station was known to have been located adjacent to such sites, unusually high levels could have resulted from this activity.

To verify that construction sites were, indeed, a significant source of airborne asbestos fibre, sampling was conducted in lower Manhattan near construction sites where extensive spraying of asbestos-containing fireproofing material was taking place. Table 3 shows the results of this sampling, and demonstrates that spray fireproofing can contribute significantly to asbestos air pollution. In some instances, chrysotile asbestos levels of approximately 100 times "background" are observed.

Table 3. Chrysotile air levels near spray fireproofing sites

Sampling location	Number of samples	Asbestos air level in 10^{-9} g/m³	
		Range	Average
⅛–¼ mile	11	9 –375	60
¼–½ mile	6	8 – 54	25
½–1 mile	5	3.5– 36	18

RESULTS OF SAMPLING AND ANALYSIS OF RIVER WATER

During the period March 1971 to March 1972, water samples were taken each month from a series of stations located on the Juniata River in Pennsylvania and on the Connecticut River which flows through the New England states. The stations on each river ranged from the upper to the lower reaches of the river. The purpose of the study was to establish the general levels of asbestos fibre which might be encountered in river waters in the eastern USA, and to determine if there were any significant seasonal variations in those levels.

Table 4. Chrysotile content of Eastern USA river water

Sample source	Number of samples	Range in micrograms per gallon	Average in micrograms per gallon
Juniata River			
Breezewood, PA	13	0– 9.2	2.2
Newton-Hamilton, P.A.	13	0– 6.2	2.2
Lewistown, PA	10	0–15.0	4.7
Amity Hall, PA	10	0–14.8	3.5
Connecticut River			
Canaan, VT	13	0–13.9	2.6
Littleton, NH	13	0–13.8	2.6
Lebanon, NH	13	0– 2.6	1.3
Greenfield, MA	13	0–23.5	5.1
Middletown, CT	12	0–14.5	5.9

The results of the study are shown in Table 4. In all the samples in which chrysotile asbestos was found, the fibres were electron microscopic in size, and many of them were probably suspended in the water as individual fibrils. There was no statistically significant seasonal variation in the amount of fibre found in the rivers. The numerical concentration values reported in Table 4 should be considered as indicative of the order of magnitude of fibre load in the water rather than as exact values. The extremely small quantity of fibre observed in the samples (e.g., 1 μg/gal, equivalent to less than 1 part per

billion) makes quantitative analysis very difficult. The fibre in the river water could have resulted both from man's activity and from natural sources. In any case, the amount found is relatively small, even though the lower reaches of these rivers pass through areas of industrial activity.

SUMMARY

Chrysotile asbestos has been found to be present at concentrations of nanograms per cubic metre in the air of major USA metropolitan centres. Similarly, water sampling demonstrates the presence of chrysotile at concentrations of micrograms per gallon in two eastern USA river systems. The sources of these environmental levels have not been determined, but they could be both the result of man's activities and from natural sources.

Discussion summary

V. TIMBRELL [1]

FIBRES IN TISSUE

There was a useful exchange of experience on the examination of tissue for the presence of asbestos fibres. It was agreed that where there had been industrial exposures plenty of fibres were found in lung sections. If amphibole asbestos was present, fibres were readily detectable by light microscopy. Chrysotile fibres were difficult to see in normal lung sections but were easily seen in sections treated by low-temperature incineration. In a UK study it had been found that the number of uncoated fibres was roughly proportional to the extent of fibrosis. On the other hand, the presence of a very large number of asbestos bodies did not correlate with fibrosis. In some post-mortem samples where the individual had died from other causes many bodies had been found, but few fibres and no fibrosis. In other cases where many fibres and relatively few bodies had been observed there was advanced fibrosis. The parameters of the dust that could relate to mesotheliomas and carcinomas are not known.

Fibres seen by light microscopy in lung tissue from the general population are only a very small percentage (usually less than 1%) of those seen by electron microscopy, and comparison with industrial exposures requires electron microscope examination of all samples. A carbon extraction technique has been developed for this examination.

SAMPLING METHODS

The discussion showed that counting fibres in membrane filter samples is the most widely used method for assessing concentration of asbestos dust in industrial environments. Some doubt was expressed about the relevance of counting fibres (5–100 μm in length) by light microscopy, especially as this type of examination would not detect the fine fibres. In a UK study, however, it had been found that such counts were generally in proportion to the fine material down to electron microscope levels.

A plea was made that laboratories reporting fibre counts should also give data on the reproducibility achieved by their observers and the degree of agreement with other laboratories. Such information was essential to establish the reliability of published data on asbestos dust levels before these were used to construct dose-response relationships. The need for laboratories to get together on this problem had been illustrated by the results of a counting exercise on a series of 30 membrane filters, in which the re-test correlation coefficient of 0.98 obtained by one laboratory dropped to 0.22 in a comparison with another laboratory. That a marked improvement could be achieved had been demonstrated in an exercise between a number of European laboratories, with excellent agreement in all cases.

Attempts are being made to overcome the tedium of fibre counting by turning to automatic methods which are expensive but can handle a large number of samples. A recent observation that airborne amphibole fibres align in a magnetic field is being used to prepare samples in which the fibres are conveniently sized and counted. Identification of the fibre types present is assisted by differences between the orientations assumed by amosite and crocidolite fibres and the non-alignment of chrysotile fibres.

Infra-red and X-ray diffraction techniques have been developed to the point where they can evaluate 10 μg quantities of asbestos in 0.1 mg samples. But there are objections to present gravimetric methods of sample assessment, including their inability to

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differentiate between fibres and other particles and to select fibres using the same criteria as are used in fibre-counting techniques. There is little evidence on the importance of these objections. The discussion indicated that interest in the simplicity and other advantages of gravimetric methods, especially in view of the recent advances in analytical methods, will result in considerable effort being devoted to overcoming the present difficulties.

Sampling instruments capable of collecting a million litres of air in a reasonable time are being investigated for assessing asbestos in the general environment. Analytical methods, including X-ray diffraction and electron microscope techniques, are being developed to measure asbestos concentration as low as 10^{-10} g per cubic metre, even in the presence of excessive amounts of other airborne solids.

Greater use of personal samplers was advocated. The samples should be taken over hours to cover the variation of dust level encountered in the process under examination, and taken at reasonable intervals to cover long-term variations in dust levels due to changes in quantity of fibre or performance of the dust control equipment.

Data from a current study on comparative sizes of airborne fibres in South African amphibole mines were presented to illustrate the need for increased use of the electron microscope in research on asbestos dusts. Evidence had been obtained (from examination of thermal precipitator samples) supporting an earlier observation (on milled rock specimens) that the crocidolite mined in the north-west Cape produced much finer fibres than both the crocidolite and amosite mined in the Transvaal. This difference, which may be a factor in the very different incidence of mesothelioma rates in the two areas, had not been evident in light microscope examinations which failed to detect the finer fibres. Whereas light microscopy may be adequate for dust studies relating to asbestosis, research on mesotheliomas probably requires different sampling strategy and sample assessment involving electron microscopy.

CRITERIA FOR ENVIRONMENTAL DATA AND BASES OF THRESHOLD LIMIT VALUES

Chairman — S. Speil

Rapporteur — V. Timbrell

Environmental data in industry

S. HOLMES [1]

INTRODUCTION

The theory and application of hygiene standards has been discussed by Berry[2]. He has outlined the hygiene standard for chrysotile established by the British Occupational Hygiene Society in 1968, being careful to point out that the data was based on experience with a group of men having a minimum of 10 years' exposure in a particular asbestos textile mill at a particular time (June 1966). No account was taken of men who had worked for the minimum period but who had left the industry before that date. Thus the information, although the best available at the time, was, to say the least, scanty for the purpose, and some of us who were associated with it have become increasingly concerned with the authority with which it has become invested in the international field. The time has therefore arrived for an up-to-date re-appraisal of the original data and for the study of new data which has since become available. Unfortunately, any retrospective study will be unlikely to meet all the conditions necessary to calculate a reliable standard, and therefore a prospective study must be undertaken, properly designed and directed towards the collection of the right information.

THE MEDICAL ASPECT

The BOHS standard for chrysotile was established at a dose level designed to limit to 1% the risk of contracting the first signs of asbestosis. The criterion used by Knox, who worked at establishing this standard, was the presence of persistent basal rales. He also looked at X-ray changes, using the techniques available at the time, and noted that while all subjects exhibiting X-ray changes also had persistent rales, some subjects had rales without X-ray changes. He therefore concluded that rales gave the earliest demonstrable sign.

Since that time, X-ray techniques have improved, and more precise systems of classification have been agreed and adopted. Studies of lung function in asbestos workers have also been made, in the hope that changes in one or more of the measured parameters, e.g., gas transfer factor, might be indicative of early asbestosis. It is therefore no longer likely that basal rales will continue to be accepted as the earliest demonstrable sign, and medical opinion could well lean towards X-ray changes or lung function changes or even towards a combination of all three. A decision would have to be made on this point, but it is interesting to note that the main prop of the Medical Surveillance Scheme for Asbestos Workers, recently introduced by the Medical Services Division of the Department of Employment, is the examination of X-ray films. Furthermore, Smither (1972 [3]) reports that in a study of ten cases of asbestosis with minimum disability, all but two cases revealed X-ray changes as the first indication of abnormality.

MEASUREMENT OF EXPOSURE

As the purpose of a hygiene standard is to control the risk to the individual, the exposure must be assessed by means of personal samples. This form of sampling in the asbestos industry has been used only recently; but it is becoming clear that samples of

[1] Asbestosis Research Council, Turner Brothers Asbestos Company Limited, Rochdale, Lancashire, UK.

[2] See p. 145 of this publication.

[3] Unpublished data.

this type, taken over 4 or 8 hours, can in many instances be different from the former "static" samples taken in the vicinity of a machine or process, although this is, of course, dependent upon whether or not the job calls for the worker to remain effectively in one place relative to the machine. Continuous sampling is to be preferred, but a time-weighted average based on a series of shorter period, discontinuous samples is acceptable provided that the latter are truly representative and that they cover the variations in dust level encountered in the process. Experience has shown that for most processes a sample covering a 4-hour period will be adequate to take account of such variations, although the recently introduced US standard calls for an 8-hour average. Samples should be taken at reasonable intervals to ensure that possible long-term variations in dust levels due to changes in asbestos fibre quality or to variations in performance of dust control equipment are adequately covered. The proposal under the D of E Medical Surveillance Scheme to base the exposure of the worker on a single dust sample taken at two-year intervals can hardly be regarded as satisfactory, but the industry itself may be able to supplement this from its own measurements.

AREAS TO BE COVERED

In establishing standards related to the asbestosis risk, it is advisable to choose situations, such as factory operations, where dust concentrations do not fluctuate widely. Thus, it would be unwise to measure processes experiencing high peaks of concentration because too little is still known about the effects of such peaks, albeit for short periods of time, on the normal lung clearance mechanisms.

To assess possible differences in the effects of the different types of asbestos, the areas of study should be chosen where the exposure is to one type only—mixed exposures should be avoided. Even with one type of asbestos, there may be variations between deposits from different mining locations which could have a bearing on their biological effects. It is therefore advisable to keep records wherever possible of the origin of the raw material.

FORM OF THE STANDARD

In establishing the BOHS standard for chrysotile, it was assumed that asbestosis was a dose-related disease, and that therefore the adoption of a standard in the form of a limitation on total integrated exposure (concentration × time) was a reasonable proposition. Nothing has occurred in the meantime to change this viewpoint, and it is recommended that any revision of this standard, or of the standards for the other types of asbestos, should be calculated on the same basis. It is further recommended that the standards should continue to be based on asbestos fibre counts using the membrane filter method, but that data based on mass estimation should also be accumulated when a satisfactory sampling technique has been devised.

In adapting the standard for control of the hazard, the concepts of total integrated exposure must be upheld and, within limits, maximum allowable concentrations should be derived from it, based on the maximum length of time the person being protected is likely to be exposed. It is well known that the threshold limit value of 2 fibres/ml was calculated from the BOHS chrysotile standard, assuming an occupational exposure of 50 years. While this may be desirable for persons working full-time with asbestos, it is probably too tight a standard for many asbestos users who handle the material only intermittently. The possible effects of intermittent high dust concentrations have already been referred to; it is therefore recommended that whatever threshold limit value (TLV) is stipulated (based on the average concentration over, say, 4 hours) exposure of the worker to peaks higher than five times the TLV (measured over 10 minutes) should be avoided.

LIMITATIONS

The foregoing recommendations are for a standard designed to control asbestosis, but the same standard will also serve to control asbestos-induced lung cancer, accepting the principle that in the absence of any other agency lung cancer occurs only in the presence of asbestosis. As Berry[1] points out, the position with regard to pleural and peritoneal mesothelioma is uncertain, and it can only be hoped that the continuing accumulation of data relating the morbidity of asbestos workers exposed to the different types of asbestos may throw further light on the problem.

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[1] Unpublished data.

THE PRESENT POSITION

It is now six years since the presentation of the data on which the BOHS standard for chrysotile is based, and, in collaboration with Dr Lewinsohn, arrangements are being made to have the information from the same factory brought up to date. Unfortunately, although new subjects will have come within the orbit of the study, others in the original study will have meanwhile left the industry, and the results might still be biased. Under the D of E Medical Surveillance Scheme, however, provision is made for a follow-up of workers after they leave the industry, and provided adequate dust monitoring arrangements are made the type of data required for the establishment of valid hygiene standards will become available in due course.

SUMMARY

The need for hygiene standards for airborne asbestos dust based on wider studies than were available to the BOHS in 1968 is emphasised. An outline is given of the type of investigation which should be carried out by industry to provide the necessary information on the relationship between morbidity and exposure to dust in asbestos workers.

REFERENCE

British Occupational Hygiene Society (1968) Hygiene standards for chrysotile asbestos dust. *Annals of Occupational Hygiene*, **11**, 47–69

Environmental data in mining

G. W. GIBBS [1] & R. S. J. DU TOIT [2]

There are six main varieties of asbestos, five of which are or have been of economic importance to various countries. Chrysotile, which accounts for approximately 93% of all asbestos produced at the present time, is mined mainly in Canada and the USSR, with smaller deposits in the Republic of South Africa, Southern Rhodesia, Cyprus, Italy and the USA. Crocidolite, which is mined in South Africa, Bolivia and until 1966 in Australia, accounts for about 3.5%; amosite, which occurs in economic amounts only in South Africa, 2.4%; and anthophyllite and tremolite for 1.1%.

Epidemiological information on the health effects of individual members of the asbestos group can be obtained only by studies of workers who are uniquely exposed to a specific variety. It may, therefore, be misleading to group health effects until it is known that all varieties, with their accessory contaminants for the same dose, are equally capable of producing the same effects in man. In the manufacture of asbestos products and in the insulation trades, workers have been exposed to more than one variety, fibre has come from different sources, and other fibrous and non-fibrous materials have been used. Thus, mining and milling environments appear to be the only ones where the effects of the various fibre types can be studied separately. Even here, exposure is complicated by rock dust, accessory minerals, and organic and inorganic contamination resulting from processing methods. The objectives of this paper are to define the mining and milling environments of asbestos producers throughout the world, to outline the current state of dust measurements and controls, and to identify sources of information of possible value in studies of the biological effects of asbestiform minerals.

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WORLD DEPOSITS

The distribution of world deposits has been reviewed by Bowles (1959), Sinclair (1955) and Hendry (1965). The main deposits, with approximate years of operation, are shown in Table 1.

GEOLOGY OF DEPOSITS

The geology of individual asbestos deposits influences mining methods and the quality of dusts to which miners and millers are exposed. Because there is a wide variation in rock types, structures, accessory minerals and fibre within even a small geographic region, it is only possible here to outline the salient features of certain deposits.

Chrysotile

Chrysotile occurs in serpentinised peridotites, dunites, pyroxenites and certain other basic or ultrabasic rocks (e.g., in Quebec and South Africa). The fibre occurs as vein or slip varieties and occasionally as mass fibre. Chrysotile is also found in serpentinised dolomites (South Africa). Another mode of occurrence is as coalinga fibre, which consists of soft, powdery agglomerates (West California and Yugoslavia). In serpentinised dolomites and peridotites, the fibre is often structurally controlled and local acid igneous intrusions, hydrothermal activity, faulting and shearing have modified the ore body. In Quebec, talc, mica and brucite are typical contaminants of some deposits.

Table 1. World asbestos deposits

Country	Year first mined on modern economic scale	Country	Year first mined on modern economic scale
	Chrysotile [a]		Crocidolite
USSR	1885	South Africa	
Canada	1878	NW Cape	1893
Swaziland	1937	Transvaal	1920
Transvaal (S Africa)	1906	Australia	1943–66
Venezuela	1953	Bolivia	
Italy			Anthophyllite [b]
China	1914	Finland	1918
New Zealand	1941	Portugal	before 1943
Japan	1939	Egypt	1950
Cyprus	1904		Tremolite
India	before 1917	Italy	1865
USA		India	1921–32
Southern Rhodesia	before 1908		Amosite
		South Africa (Transvaal)	1925

[a] Small amounts of chrysotile have been mined in Mexico (1943), Brazil (1939), Chile (1945–50), Colombia (1934), Czechoslovakia (1953), France (1934–38), Spain, Korea (1930–45), Turkey (1930), Bechuanaland (1952) and Morocco (1940–44).
[b] Small amounts of anthophyllite have been mined in many countries, including Madagascar and South Australia.

Amosite

The only economically exploitable deposits of amosite occur in the Transvaal in South Africa. The fibres occur in banded ironstones within dolomites. It is significant that amosite occurs in the same area as Transvaal crocidolite; furthermore, these two minerals can occur in such proximity as to be mined together.

Crocidolite

Crocidolite from Cape Province and Transvaal in South Africa occurs in banded ironstones, and fibres range in length from less than $\frac{1}{2}$ inch to 2 inches or more.

Anthophyllite

Anthophyllite occurs in lenticular forms of mass fibre in metamorphosed ultrabasic rocks, and in this paragenesis it is commonly associated with talc.

Tremolite and actinolite

These minerals are found in impure limestones and dolomites which have undergone recrystallisation. Tremolite is also a common contaminant of some talc deposits.

The geology of the major deposits of South Africa, Southern Rhodesia and Canada have been described in detail by Biljon (1963), Laubscher (1963), Genis (1963), Cillier & Genis (1963), Allen *et al.* (1957), Riordon (1957, 1962a, 1962b).

MINING METHODS

Depending on local structures and economics, chrysotile is mined by open-pit or underground methods. In Quebec, there are in operation three underground mines and five open pits; however, prior to 1930 all production was from open-cast workings. This appears to have been true for most countries. Chrysotile is mined using open-pit methods in Cyprus, the USSR and from most deposits in South Africa. In contrast amosite and crocidolite are produced from underground. Open-pit methods have differed mainly in kinds of explosives, means of transportation, types of drill and methods of dust control. In some Quebec companies, dust is collected at source during primary drilling using cyclones and filter bags. In Rhodesia dust from the drill hole is discharged at a point down-wind of the driller, and in South Africa wet drilling is practised.

In the past all mining operations were similar; only high grade ore was mined and dust was little controlled. Even today certain workers may be exposed to high dust concentrations during secondary drilling.

MILLING METHODS

Ore from the open-pit and underground mines is generally crushed and dried before transportation to

the mill. In South Africa, up to 1958, drying was achieved by letting rock lie in the sun; now some mines use furnaces. In Cyprus the mine worked only during the dry season. In Quebec, fibre has been dried for many years by coal- or oil-fired rotary and vertical dryers, and during early mining operations by steam pipes.

The process for removal of fibre from ore is essentially the same for all varieties. Ore is fractured mechanically and fibre is aspirated from the rock on a series of screens, then graded, tested and bagged. The recovery of fibre, particularly crocidolite and amosite, from the host rock requires the physical separation and rejection of a large proportion of waste. The hardness of the host rock in which crocidolite and amosite occur leads to considerably more mechanical abrasion during processing than is encountered with chrysotile, thus trace metal contamination is likely to be higher.

In the past, and even today at some mills, exposure to dust results from uncovered screens, open discharge points, and from inadequate ventilation for dust control. In the chrysotile milling industry of Quebec, efficient methods of dust control were first introduced in the early 1950's, and air from the mill now passes through high efficiency filter bags and is returned to the mill; heat generated by the machinery thus keeps the mill warm during the winter. Bagging, which was at one time one of the more dusty occupations, has improved to the extent that this work area is now one of the cleanest in the mills. Maintenance personnel who may be required to work in all areas of plants often receive dust exposure in excess of those working routinely in the mill.

DUST MEASUREMENTS PAST AND PRESENT

There is little published data on dust conditions in mining and milling environments. In Cyprus, dust measurements using short- and long-running thermal precipitators were first made in 1963. Spherical particles of 0.5–5.0 μm in diameter and fibres up to 50 μm in length were counted using an oil immersion microscope (2 mm objective) and a microprojector (Kronides, 1972 [1]). It was estimated that dust concentrations in the mills from 1924 until 1962, when new mills were built, may have reached 3000 particles per cubic centimetre (ppcc). In Quebec mills airborne concentrations were measured on an almost annual basis from 1949 by the same observer (Gibbs & Lachance, 1972). These measurements were made using the midget impinger and were counted at 100× magnification. Prior to 1950 dust levels in certain mills exceeded 100 million particles per cubic foot (mppcf) (3530 ppcc), and in some areas reached 200 mppcf. Dust levels in the better mills are now less than 2 mppcf (70.6 ppcc).

In South Africa, the konimeter has been used since the early days of asbestos mining and is still in use (du Toit, 1970). The thermal precipitator was introduced in 1940, and the membrane filter method in 1970. The konimeter is used to obtain snap samples and the thermal precipitator for systematic dust surveys. The number of konimeter samples taken was 1 per annum per 26 dust-exposed persons (5000) in 1940, and 1 per 13 in 1950 and 1961; however, systematic dust surveys were done at irregular intervals varying from a few to 9 years. Since 1965 thermal precipitator samples have been taken every two years with a sampling frequency of one sample per 72 persons employed. A summary of available mine and mill dust concentrations is given in Table 2. It must be borne in mind that these data were obtained at different locations, using different methods and were usually collected for control rather than for epidemiological purposes. The concentrations are however based on sufficient numbers of samples to reflect the general dust conditions.

Comparisons of dust levels in the various mining industries are not possible since different methods of measurements have been used and the conversion factors are not known. However, in spite of fluctuations, yearly levels are decreasing in all mines and mills. In South Africa, the mean dust level at the surface for chrysotile mines was as high and higher than that for crocidolite or amosite mines. The underground total dust levels were lower than mill dust levels in Canada and South Africa.

POPULATION AT RISK

The total mining population in Quebec at present is about 6000, and approximately 30,000 persons have been employed in the industry since 1878. In South Africa the number of persons employed in the mining and milling industry has increased from about 5000 in 1950 to about 20,000 in 1970. The average

[1] Personal communication.

Table 2. Dust concentrations in mines and mills

Year	Fibre (≥ 5 μm) plus particles per cm³											
	Republic of South Africa [a]								Canada [b]		Cyprus [c]	Italy [d]
	Crocidolite				Amosite		Chrysotile		Chrysotile		Chrysotile	Chrysotile
	Cape		Transvaal		Transvaal				Quebec		Mills	Ballangero
	S	U	S	U	S	U	S	U	Mill	U		
1940												
41												
42												
43	2687	1139			1458	350	423	285				
44												
45												
46												
47												
48	1742	483			571	315	1734	217				
49									1094			
50									1553			
51									1518			
52									741	339		
53	1036	404			646	445	575	220	1623	777		
54									777	494		
55									706			
56									706	106		
57									494			
58									636			
59									318	56		
60									212	42		
61									93			
62									234	268		
63									494	212	1218	
64									219		1045	
65	375	370	633	197	495	130	1684	196	204		1183	
66									242		624	
67										296	680	
68	250	192	246	167	522	161	592	198			402	Range
69											584	60–2850
70	273	146	110	96	354	150	226	138			1190	
71											868	

[a] By konimeter until 1965, thereafter by thermal precipitator—mean concentrations.
[b] Midget impinger—median values.
[c] Thermal precipitator—median values.
[d] 380 counts—membrane filter.
S = Surface, U = Underground.

number of persons employed in the Rhodesian mines between 1963 and 1967 was 8336 (Gelfand & Morton, 1970). In Finland 1092 persons had worked for more than 3 months in the anthophyllite mines between 1937 and 1967 (Kiviluoto & Meurman, 1970); and the work force in the Australian crocidolite mines was reported as 300–400 (McNulty, 1970).

QUALITATIVE FACTORS

The recent implementation of threshold limit values based on fibre counts has caused some consternation in the mining and milling industries. Virtually all past measurements have been particle plus fibre counts, and in the USSR, airborne dust concentrations have been determined gravimetrically. What do total airborne particle counts mean in terms of fibres ≥ 5 μm in length? Studies in textile plants found virtually no correlation between total particle counts using the midget impinger and fibre counts made using the membrane filter technique. On the basis of extremely limited data it appears that it may be possible to obtain a conversion factor for the Quebec mills if each work area is considered separately. Calculation of a conversion factor based on the fibre content of airborne dust agreed well with limited side-by-side midget impinger-membrane filter comparisons. The airborne fibre content (fibres ≥ 5 μm) in three chrysotile mills ranged from 2 to

9% depending on location, and the overall mean values agreed well with those for South African chrysotile mills (Table 3). The higher percentage of visible fibres in airborne dust in the Transvaal may reflect the larger mean fibre diameters in this area compared with those found in the Cape, rather than a real difference in percentage of airborne fibres.

Table 3. Percentage of fibre in airborne dust

Fibre variety and location		% Fibres ⩾ 5 μm
Cape crocidolite	Surface	7.0
	Underground	2.0
Transvaal crocidolite	Surface	11.0
	Underground	4.0
Amosite	Surface	16.0
	Underground	2.0
South African chrysotile	Surface	4.0
	Underground	2.0
Quebec chrysotile [a]	Open pit	1.7
	Crusher	2.4
	Mill	3.0
	Bagging	4.6

[a] Based on 81 measurements at 3 mines only.

FIBRE LENGTHS

Information on airborne fibre lengths is limited. In South African mills the amosite and crocidolite fibres in the size range ⩾0.5 μm appear to be longer than those found underground, and on average they are twice as long as chrysotile fibres, as seen on thermal precipitator slides using optical microscopy. In a limited number of thermal precipitator measurements made in 1941 and 1972, no change in mean fibre length was observed (Table 4).

PROPORTION OF AIRBORNE MASS WHICH IS RESPIRABLE

Table 5 shows the percentage of airborne respirable dust in South African mines measured with BAT I with efficiencies as described by Breuer (1963) and using a Cassella 113A dust sampler. The comparable data for Quebec chrysotile mills, based on extremely limited observations, using Cassella Hexhlet samples was between 14 and 25%.

Table 4. Mean fibre (5–100 μm) lengths by thermal precipitator

Year	Type of fibre		No. of samples [a]	Length (μm)	
				Range	Mean
1941	Amosite	Surface	6	12–15	13.7
1972	Amosite	Surface	3	13–14	13.8
		Underground	6	9–11	10.1
	Transvaal crocidolite	Surface	9	8–13	9.9
		Underground	11	8–25	11.0
	Cape crocidolite	Surface	6	9–14	11.7
		Underground	13	6–20	9.9
	Chrysotile	Surface	6	7–11	9.8
		Underground	7	7–16	10.4

[a] Two observations per sample.

Table 5. Percentage of respirable dust in asbestos mines and mills

Type of dust		BAT I			Cassella 113A	
		No. of samples	Range %	Mean %	No. of samples	Range %
Cape crocidolite	Surface	5	1–28	11	2	31–40
	Underground	9	7–87	38		
Transvaal crocidolite	Surface	3	6–48	25		
	Underground	6	3–38	18		
Amosite	Surface	1		31	2	10–30
	Underground	2	2–4	3		
Chrysotile	Surface	3	4–6	5	1	19
	Underground	8	7–95	64		

TRACE ELEMENTS ASSOCIATED WITH FIBRE

The trace metal contents of airborne fibre in Canadian chrysotile mills have been reported (Gibbs, 1971). They tend to be concentrated in the respirable fraction of dust and are thus likely to be present in fibre dispatched for manufacture. The importance of trace metals is discussed elsewhere in this symposium.

MINERALS ASSOCIATED WITH FIBRE

Exposure in the mining industry is not only to fibre, but to fibre plus accessory minerals, host rock, etc. Indeed, the structures of fibres from various areas may differ (e.g., Indian chrysotile contains 80% orthochrysotile; while fibre from one mine in Quebec contains only 7%, the balance being of the clino variety). It has recently been demonstrated (Gibbs, 1972 [1]) that in Quebec pleural calcification is almost certainly related to a mineral contaminant of fibre from one small area. Samples of respirable airborne dust (BAT I) from some South African mines contain amounts of quartz ranging from 3% in underground chrysotile mines through 8% in amosite and 7% in Cape crocidolite mines to 21% in the Transvaal crocidolite mines. The quartz content of airborne dust at the surface (samples not acid-treated) was 5%[2], 10%, 7% and 17%, respectively. The levels of quartz in the Transvaal crocidolite should be considered a factor in lung fibrosis. The quartz content of fibre from other countries has not been reported.

CURLINESS OF FIBRE

Limited studies of airborne fibres in three chrysotile mines and mills in Quebec suggested no difference in the relative proportions of straight to curly fibres in mines producing harsh, medium-harsh and soft fibre; the median percentage of curly fibres was approximately 50%. Crocidolite samples taken with membrane filter and thermal precipitator in the mines and mills of the Cape and Transvaal contained 0–16% of curly fibres, however, the degree of curliness was substantially less than in Quebec fibre.

ORGANIC CONTENT

The concentrations of polycyclic aromatic hydrocarbons in airborne samples of dust collected in chrysotile mills in Quebec were less than those encountered in the urban atmosphere of Montreal; however, organic contamination of fibre can occur during mining and milling, and chrysotile at one mill contained 20 mg organic matter/100 g fibre. "Virgin" specimens of chrysotile from Quebec yielded less then 5 mg of benzene-extractable material per 100 g fibre, which is considerably less than that reported by Harington (1965) in specimens of crocidolite and amosite from South Africa. It seems unlikely that the trace amounts of polycyclic aromatic hydrocarbons in the various fibre types at source are important; however, the role of the total organic contaminants remains unknown.

THE FUTURE

Although dust levels in mines and mills have been decreasing, there is still room for improvement. There is an urgent need for an inventory on an international scale of persons at risk in all mining and milling industries, which would facilitate planning of epidemiological studies of the effects of fibre at source. Both for purposes of international comparison and in order for experience gained in one mining or industrial operation to be applicable to others, dust assessment must be standardised. The applicability of a fibre standard to an industry where all criteria for control and all epidemiological evidence has heretofore been based on particle counts should be carefully considered before current methods of evaluation in the mine and mill environments are discarded. Qualitative aspects, including physical parameters of the airborne dust clouds in each mining, milling, manufacturing and utilisation industry, should be carefully documented.

[1] Unpublished data.
[2] This value is unexpectedly high, and the measurement is to be repeated.

SUMMARY

(not provided by authors)

Mining and milling environments are probably the only ones where the effects of the various asbestos fibre types may be studied separately, although exposure is often to other materials as well. Comparisons are made between the mining and milling environments of asbestos producers throughout the world, and the current states of dust measurements and dust control are described.

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Hygiene standards—theory and application

G. BERRY [1]

I will be considering certain aspects of a hygiene standard for asbestos dust. Although this paper is specifically concerned with asbestos, the first part, on the theoretical aspects and associated practical applications, may also apply to other contaminants to which occupational exposure occurs.

The term "hygiene standard" is being used to mean a level of atmospheric contamination by dust which is considered acceptable as far as the hazard to health of exposed workers is concerned. This level is frequently termed the "threshold limit value", but I will avoid this term as it implies the existence of a threshold below which conditions are completely safe. In fact the term as used is not meant to imply this, as is indicated in the Preface to the list issued by the American Conference of Governmental Industrial Hygienists (Committee on Threshold Limits, 1969), where threshold limit values are said to represent conditions under which it is believed that *nearly* all workers may be repeatedly exposed day after day without adverse effect. Therefore the term "hygiene standard" seems to be preferable since it is devoid of any particular connotations.

THEORY

A hygiene standard must be based in part on the dose-response relationship, i.e., the relationship between exposure to asbestos (the dose) and the risk of an adverse effect on health (the response). I will restrict attention to cases where the response is quantal, i.e., where a disease state, for example, asbestosis, can definitely be said to be present or not. The response is measured by the proportion of people at risk who develop the disease, and the dose-response relationship shows how this proportion varies with different amounts of exposure.

There are two types of dose-response relationship, which must be differentiated. In the first, the concept of a threshold is invoked, i.e., it is assumed that there exists some non-zero dose below which the risk is zero. In the second, it is assumed that any dose, however small, involves a corresponding risk. In making this assumption it is not necessary to reject the threshold concept outright; it would still hold if each individual had his own threshold but if these thresholds varied among individuals, with the possibility that for any dose, however small, there were individuals with thresholds still lower (Mantel, 1963).

In choosing a hygiene standard the use of the concept of a threshold results in considerable simplification. For if a non-zero threshold were to exist, and if its value were known, one could specify this value as the hygiene standard, and this standard would be completely safe. But if the second form of relationship were appropriate, it would not be possible to specify any value, except zero, as completely safe. The choice would then lie between prohibiting use of the material, or deciding on some level of effect as acceptable. This choice will be considered later, after first establishing that the concept of a non-zero threshold cannot be upheld in practice.

There are two main objections to basing a hygiene standard on the assumed existence of a threshold; both are practical objections, which would apply even if the concept were considered beyond dispute biologically. First, the value of this threshold could never be determined from epidemiological data; for example, if at a certain dose there were no cases of disease out of 1000 individuals at risk, then it could only be stated with reasonable certainty that the risk was less than 3 in 1000. Secondly, even if the threshold value were known it would not be possible to deduce from a limited number of dust measure-

[1] MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, UK.

ments that a worker would never be exposed to higher concentrations. Thus, it can only be shown that the risk of an exposed worker being affected is small (British Occupational Hygiene Society, 1968).

Hence, assuming that asbestos is an essential material for which there is no effective substitute, it is necessary to define an acceptable level of risk. This is a very difficult problem since it inevitably involves balancing the risks to health against the benefits of the material, and against the consequences of demanding excessive dust reduction (Brues, 1971; British Occupational Hygiene Society, 1968). Indeed in a slightly different situation, because he found that toxicologists were unwilling to follow Gaddum (1956) in nominating a tolerable effect, Williams (1971) proposed a statistical technique to overcome the problem. However, as this technique accepts a dose to be safe unless there is positive evidence that it is not, his approach ignores rather than overcomes the problem. This attitude had been criticised by Mantel & Bryan (1961) and by Gross *et al.* (1970), who wrote "evidence like this is really lack of evidence". They also argued that the establishment of safe levels dependent on the threshold level postulate was a dangerous procedure, and they therefore adopted the approach proposed above which they called the "risk-limited approach".

There are two stages in defining an acceptable dose: first, it must be decided what degree of disease, or what sign or symptom, to use as an index; and, secondly, it must be decided what incidence of this index is acceptable. These two decisions are interrelated, the milder the index, the higher the acceptable incidence. Moreover, if (*a*) the population at risk is subject to regular medical surveillance to identify those who have developed the index, (*b*) it is practical to remove those so identified from further exposure, and (*c*) removal from further exposure at this stage will be effective in preventing a case from progressing to more serious stages, then the acceptable incidence may be higher than otherwise. It is desirable to use as an index a mild condition, preferably one which is not itself indicative of ill health but is an early predictor of impending ill health; however, Hatch (1971) criticises a tendency to assess the safety of conditions by relying on some index of reaction with no known relation to eventual ill health.

DATA REQUIREMENTS

In order that the dose-response relationship may be estimated without bias, the data must come from a representative sample of a population similar to the population about which one wishes to make inferences. The population sampled may consist of all workers joining a factory during a particular period; or it may be more efficient to have a stratified sample, i.e., made up of sub-samples from each of several strata, each stratum representing a different level of exposure. This condition is difficult to fulfil since it requires information about workers who have left the industry.

APPLICATION

The threshold limit value adopted by the American Conference of Governmental Industrial Hygienists

Table 1. Data on men employed in chrysotile asbestos textile factory

Years employed	Number exposed	Mean dust concentration [a] (fibres/cm³)	Cumulative dose (fibre years/cm³)	Number (%) with basal rales
Group 1				
10–	58	10.9	131	1 (1.7)
15–	72	11.9	202	1 (1.4)
20–	29	13.5	296	4 (13.8)
25–	46	14.5	392	8 (17.4)
30–	6	15.2	486	1 (16.7)
Group 2				
10–	22	3.4	41	0
15–	20	3.6	60	0
20–	10	3.8	84	0
25–	20	4.0	107	1 (5.0)
30–	7	4.1	131	0

[a] Fibres/cm³ in terms of membrane filter samples. A "fibre" is a particle longer than 5 μm and having ratio of length to breadth greater than 3:1.

was, up to 1969 (Committee on Threshold Limits, 1969), based on a recommendation made in a report of a survey in the US asbestos textile industry by Dreessen *et al.* (1938). They found no clear-cut cases of asbestosis in 108 workers exposed to less than 5 million particles per cubic foot (mppcf). The mean dust concentration for these subjects was about 3 mppcf so that we may deduce from the above data that the asbestosis risk at a dust concentration of 3 mppcf was probably less than 3%. This is completely different to concluding that 5 mppcf was a threshold value below which no new cases would appear; although Dreessen and his co-workers drew this conclusion only tentatively ("until better data are available").

The British Occupational Hygiene Society (1968), in reconsidering the question for chrysotile asbestos, was able to produce only one further set of data. This is given in Table 1 and consists of results obtained from two groups of men working in an asbestos textile factory in 1966 who had worked there for at least 10 years. Group 1 consists of men working in fiberising, carding and spinning; and Group 2 of those who were exposed to lower dust concentrations, in weaving and plaiting. The mean dust concentrations were estimated, and the dose was taken as the cumulative exposure, i.e., as the length of exposure multiplied by mean dust concentration. The index of response was taken as the recording of basal rales.

A dose-response curve was fitted to the data, assuming that the distribution of doses for which basal rales may be first recorded was log-normal. A satisfactory fit was obtained (Fig. 1).

It was decided to set the hygiene standard at the dose giving a risk of basal rales of 1%. This dose was estimated at 112 fibre years/cm^3, implying a concentration of about 2 fibres/cm^3 to afford the above degree of protection for exposure during a working lifetime. This estimate has an associated error, and there is a reasonable chance that the risk at this exposure is as high at 3%. Thus, to be reasonably certain (i.e., 95%) that the risk did not exceed 1%, the dose would have to be no more than 51 fibre years/cm^3.

The above data are not of the recommended sample type, since they are based on men working in a particular mill at a particular time; the group is therefore the result of selection, either voluntary or imposed, since some workers had left the industry. If the men who left differ in their susceptibility to basal rales from those who stayed, then the above conclusions are biased accordingly.

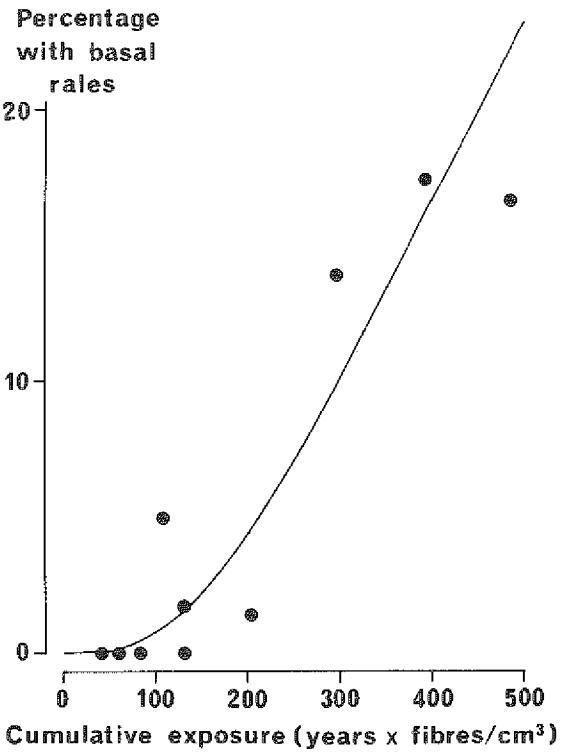

Fig. 1. Dose-response relationship for prevalence of basal rales in a chrysotile asbestos factory.

DISCUSSION

There is a necessity to specify a hygiene standard for asbestos in order to protect the health of those occupationally exposed. This involves facing up to the difficult question of deciding what risk should be accepted, and since this is a decision which may be made only partly on medical grounds it requires the participation of others besides medical research workers and hygienists. Economic factors must be considered and some of the problems of health economics are outlined by Akehurst[1].

However the decision can be made rationally only if the relevant data are available. There is a serious dearth of data on the relationship between ill health and exposure to different levels of different types of asbestos. Thus the British Occupational Hygiene

[1] See p. 329 of this publication.

Society (1968) was compelled to base its standard on only one set of data.

The problem is one affecting all industrial countries; and, although for social and economic reasons different hygiene standards may be appropriate to different countries, the type of data required has much in common. Data are needed representing different conditions in different countries. Whatever standards are adopted, it is important to continually review new information to ensure that conditions, as indicated by the health hazard, remain satisfactory, and if necessary to up-date the standards.

Data are also needed on the relationships among the different stages and types of asbestos-produced disease. Some evidence is available which indicates that the excess risk of lung cancer occurs mainly in those with asbestosis (Knox *et al.*, 1968; Elmes & Simpson, 1971), but the position in relation to pleural and peritoneal mesotheliomas is uncertain. Again, international cooperation is desirable to decide what responses should be recorded as indicators of effects on health, so that these information gaps may be filled for the benefit of those who are, and will be in the future, occupationally exposed to asbestos.

SUMMARY

The hygiene standard is defined as the level of atmospheric contamination by dust considered to be acceptable as far as the hazard to health of exposed workers is concerned. This standard must be based in part on the relationship between exposure and the risk of an adverse effect. It is shown that the concept of a threshold below which conditions are completely safe cannot be upheld in practice. This means that a hygiene standard can be specified only by balancing the risks to health against the benefits of using the material. The application of the theory is illustrated by two examples involving asbestos exposure. There is a paucity of data on which a rational choice of the hygiene standard for asbestos may be based.

ACKNOWLEDGEMENTS

Some of the data given in the Application section were originally published in the *Annals of Occupational Hygiene*, and I am grateful to the editor and to Dr J. F. Knox and Dr S. Holmes for allowing me to use it. The formulation of the principles given in this paper is a result of my time spent on the British Occupational Hygiene Society's sub-committee on hygiene standards for asbestos, and some of the better ideas should without doubt be credited to the members of this sub-committee. However, I take sole responsibility for the way in which they are expressed in this paper.

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Discussion summary

V. TIMBRELL [1]

ENVIRONMENTAL EXPOSURES

Data were presented which illustrated the feasibility of reducing contamination of the environment by asbestos dust. Until recent years dense clouds of asbestos dust were a regular feature of one Italian mine, but following improvements to the plant there had been a major reduction in dust levels in both the mills and the neighbourhood. Mean counts, in fibres/cm^3, were reduced to 1 in an area of 4 km^2 around the mine, 0.5 in a 7 km^2 area and 0.1 at the nearest villages.

In a survey of the ambient air in a number of US urban areas occasional alarmingly high levels were observed. These occurred in a city having four friction material production plants, the emissions from which were uncontrolled during the time of the survey.

Several examples were given of avoidable exposures to individuals from a variety of improper uses of asbestos. One instance cited was the recent importation into the US of over 200,000 women's coats made of a fabric in which 8% chrysotile had been incorporated to obtain a reduction in import duty. In a further example, the remainder of 25,000 lb of a children's art moulding compound containing asbestos had only recently been withdrawn from use in New York schools. The asbestos-producing industry had endeavoured to prevent such unwarranted uses, but it was important that such efforts should be extended to minimise environmental exposures.

OCCUPATIONAL EXPOSURES

Workmen not directly involved in asbestos applications may receive heavy dust exposures. Fitters, joiners and general labourers are not classified as "asbestos workers"; often they are unaware of the dangers of dust inhalation and do not have respiratory protection. Attention should be drawn to the many workers in the building construction industry who were still at risk of inhaling relatively high concentrations of asbestos dust.

Asbestos-cement spraying has often been documented as a significant source of environmental exposures, and from 1973 the spraying of any form of asbestos would be prohibited in the Netherlands by law. A UK study to monitor such an operation for the fire-proofing of the steel structure of a hospital building was described. The asbestos-cement spraying contractor observed the Code of Practice recommended by the UK Asbestosis Research Council. Before spraying, the outer shell of the building was complete and the windows in place. The spray operators used a pre-damping apparatus and a wet-spray process. They wore protective overalls and close-fitting respirators covering the nose and mouth. The fall-out of asbestos fibre was studied as it affected the asbestos spray workers, other workmen on the building site and neighbourhood contamination. The results illustrated the dust control that can be achieved even in such difficult conditions:

Airborne Fibre Concentration	*Fibres (5–100 μm/cm^3)*
Transferring amosite from bag to hod	2.6
Emptying hod into pre-damping drum	1.2
Loading spray machine with dampened fibre	0.8
Spraying operator	7.0
15 ft from spraying operator	0.03
30 ft „ „ „	0.01
Floor above	0.02
Floor below	0.01

No stray fall-out of fibre in the surrounding neighbourhood was observed during or after the spray

[1] MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, UK.

operation. The important point was made that whereas the fall-out of fibre in the vicinity of the spray operation was relatively small and the operatives were protected by their respirators, other workers on the building site were possibly subsequently at risk when handling the dried waste.

HYGIENE STANDARDS

The BOHS standard for chrysotile asbestos was criticised on a number of grounds:

(*a*) that it had been established on very little data;
(*b*) that it was based on asbestosis, of minor importance relative to cancer;
(*c*) that it used concentration × years as a measure of exposure.

In defence it was pointed out that the scantiness of the information on which the standard was based had been fully recognised in the 1968 BOHS report. It was considered to be the best approach at that time; no other information had become available until very recently. The BOHS report had also recommended that the standard be continually reviewed and modified if necessary as more data became available.

It was argued that the practice of basing a standard on long-term dust levels effectively implied a more stringent standard if compliance was tested by short-period sampling. In asbestos mining and milling coefficients of variation of 80% were common: this meant that for 3% of the time the dust levels were above 2½ times the long-term mean. The short-term standard should therefore be more lenient than the long-term standard.

The point was repeatedly made that there is unlikely to be a finite exposure value below which the risk of disease is zero, and hygiene standards must accordingly be based on a balance between benefits and risks. These involve social and commercial as well as medical considerations. A strong plea was made that workers' representatives be invited to participate in future conferences. Workers had shown concern about possible risks to their health and it was important that their views be heard. But it was also pointed out that the conference was not planned to cover the means of applying new knowledge to prevention. It was concerned with assessing the biological evidence. Prevention required consideration of many topics plus special experience not within the ambit of the meeting.

ASBESTOSIS IN RELATION TO TYPE OF FIBRE, DOSE, OCCUPATION AND DURATION OF EXPOSURE

Chairman — M. Navratil

Rapporteur — H. Weill

Asbestosis in chrysotile mines and mills

J. C. McDONALD [1]

Chrysotile has been used in the manufacture of simple fabrics for at least 2000 years. Deposits of the mineral fibre are widespread, and man's earliest sources were Cyprus, Northern Italy and China. Mining production in the modern sense began about 100 years ago, and world output has grown fairly steadily to some 4 million tons per annum. Of this, about 50% comes from the Soviet Union, 35% from Canada (mainly Quebec) and most of the remainder from South Africa, Rhodesia, China, USA and Italy. Some chrysotile is also produced in Cyprus, India, Japan, Yugoslavia and in several other countries. The number of persons currently employed in the mining and milling of chrysotile is not recorded but probably approaches 40,000. At least 5 times as many may have worked in the industry since it began.

This paper is limited to a consideration of manifestations of asbestosis as seen in chrysotile production workers, with special reference to the nature of the exposure-response relationship. The studies in which this is possible were made in Cyprus, Northern Italy and Quebec; these will be examined in some detail, and the conclusions drawn from them will be discussed in the light of data from Africa and the USSR.

[1] Department of Epidemiology and Health, McGill University, Montreal, Canada.

STUDIES IN CYPRUS, NORTHERN ITALY AND QUEBEC

Surveys of chrysotile mine and mill workers in these three regions have been made since 1966. The methods used in each were fairly similar; in all three, there have been some or all of the following elements: (1) registration of past and present employees with assessment of individual dust exposure; (2) follow-up of past employees to determine mortality rates by cause; (3) assessment of changes in chest radiographs using the U/C classification (UICC, 1970); and (4) measurements of respiratory function and symptoms in a sample of current workers. Information on the extent of these studies is presented in Table 1.

Working environment

A variety of instruments and methods have been used for measuring dust levels in the various mining areas, thus comparison of results is difficult. The median dust concentration in the Quebec mills, as measured recently by midget impinger was about 5 million particles per cubic foot (mppcf). Twice this

Table 1. Extent of epidemiological surveys in Cyprus, Northern Italy and Quebec

	Year	Cyprus	Northern Italy	Quebec
Mean annual dust levels and (range) in mills (mppcf)	1950	– (60–90)	– (–)	75 (35–200)
	1960		– (–)	12 (1–55)
	1970	– (18–42)	10 (3–20)	5 (1–10)
Fibre conversion factor (approx) [a]		1.0	1.3	2.3
Number of men in:				
Cohort survey of mortality		–	1098	11,207
Radiographic study		645	499	6529
Function and clinical study		–	355	1015

[a] Fibres/ml = mppcf × conversion factor.

value was recorded 10 years earlier; and the figure was 15 times higher in the 1940's, before serious efforts were made at dust control. Recent dust levels in Cyprus and Italy appear to be similar to those found in Quebec 10 years ago, and they were probably considerably higher in the past.

Dust exposure in the mines is generally much lower than in the mills, except in the immediate vicinity of drilling operations. It is, however, more difficult to estimate the exposure of individual mine workers, since fewer environmental measurements have been made, and because the work is both intermittent and varied.

The equivalence of total particle concentration to fibre counts is based on very scanty information. In the mines and mills of Quebec, the data suggest that 1 mppcf (as measured by midget impinger) is equivalent to about 2.3 fibres/ml (as measured by membrane filter), although the ratio is probably higher in the mills than in the mines. In Italy, the ratio seems to be about 1:1.3; and, in Cyprus, probably lower still. Thus, although dust exposure in the two European mines was generally higher than in Quebec, the amount of fibre exposure may not have been very different.

Radiographic changes

The X-ray findings in Cyprus and Quebec can be directly compared (Table 2), since essentially the same procedures were used for assessing exposure, reading films and statistical analysis (Rossiter *et al.*, 1972; Constantinides, 1972 [1]). Dr John Gilson, who read the Cyprus films, was one of the panel of six who assessed the Quebec films. In Table 2, it can be seen that of men aged 36–65 at the time of X-ray those in Cyprus had a similar employment history to those in Quebec but had heavier dust exposure, more than twice as much as workers at Asbestos. Taking into account these exposure differences, the rate of radiographic changes in the two areas is very similar.

Only preliminary findings, which do not include details of exposure, are yet available from the study by Vigliani [2] and his colleagues of chrysotile mine and mill workers at Ballangero, Northern Italy. Films from 474 current or past employees with more than one year's service were classified according to the U/C classification by four readers. Films were obtained for 395 workers comprising 63% of those still alive; and films for 79 ex-workers were obtained from the Insurance Institute (INAIL). The overall rate of irregular small opacities (sub-category 1 or more) was 29%, and of pleural thickening (grade 1 or more) 13%, both much higher than in Cyprus or Quebec. It was thought that this might partly be accounted for by the method of selection of the series. However, when the subjects had been divided into 4 groups according to duration and probable level of exposure, the rate for those in even the lowest exposure group (equivalent to less than 2 years' exposure in the Ballangero mill) was still 16% for irregular small opacities and 14% for pleural thickening. Further analysis and comparative reading trials will be needed to show whether the response in Italian workers is really more severe than elsewhere.

Table 2. Dust exposure and radiographic changes (%) for men aged 36–65 at time of X-ray in Cyprus and Quebec

	Cyprus	Thetford Mines, Quebec	Asbestos, Quebec
Number in study	370	3619	2508
Years since first employment	24.5	22.5	21.2
Years employed	23.4	20.1	20.4
Dust index (mppcf-years)	436	342	186
Irregular small opacities sub-category 1/0 or more	10.0%	7.6%	4.9%
Pleural thickening grade 1 or more	7.8%	6.4%	3.1%
Ill-defined cardiac outline grade 1 or more	5.4%	2.2%	1.0%
Pleural calcification grade 1 or more	5.9%	5.2%	0.4%
Ill-defined diaphragm	2.4%	3.5%	1.9%

Respiratory function and symptoms

A stratified random sample of current workers was examined in Quebec. In Ballangero, essentially the same tests were used, but the sample of 355, for which results are available, was drawn from current and past employees. No clinical survey was made in Cyprus.

In the Canadian study it was found that, after adjusting for age, height and weight, exposure affected only the inspiratory capacity portion of total lung volume (Becklake *et al.*, 1972). Forced vital capacity and forced expiratory volume (1 second) declined with increasing exposure up to about 18% in the highest exposure group. On the other hand,

[1] Personal communication.
[2] Personal communication.

neither functional residual capacity nor residual volume showed any appreciable reduction. Measurements of diffusing capacity at rest were unrelated to exposure but declined slightly on exercise. All tests were less sensitive to the effects of exposure in smokers than in non-smokers.

At the present time, it is not possible to compare these findings directly with those from Ballangero; but in both studies the results of function tests were related to the radiographic category of the subject in such a way as to permit limited comparison. The main findings are set out in Table 3, where it may be seen that the functional deficit in men with irregular small opacities of sub-category 1/0 or more, though similar, was rather greater in Quebec (Becklake *et al.*, 1970) than in Northern Italy (Vigliani [1]). This is consistent with the view that lesser degrees of radiographic change were considered abnormal by the Italian film readers.

Table 3. Percentage reduction in pulmonary function in men with irregular small opacities on X-ray [2]

	Italian workers	Quebec workers
Vital capacity	6.9%	8.5%
Forced expiratory volume (1 second)	5.2%	6.9%
Diffusion capacity (steady state)		
—at rest	2.2%	6.4%
—on exercise		
200 kg/min		7.6%
300 kg/min	2.8%	
400 kg/min		7.1%
600 kg/min	2.8%	9.5%

[2] In Quebec workers the percentages represent the reduction in function observed in men with irregular small opacities, sub-category 1/0 or more, as a proportion of the value for men with less than this. The figures for the Italian workers were derived in a slightly different way but are probably comparable.

The same respiratory symptom questionnaire was used in both studies, but the results are not yet available in the same form, and thus they cannot be compared. In Quebec, it was found that, allowing for age, a persistent cough and phlegm (bronchitis) were related to both dust exposure and to smoking (McDonald *et al.*, 1972). In contrast, breathlessness on exercise was unaffected by smoking but increased steadily with dust exposure.

Mortality

Of 3270 deaths recorded in a cohort of 10,120 employees of the Quebec mines and mills born between 1891 and 1920, 34 were certified as being due to pneumoconiosis. Exposure varied among members of the cohort from minimal and short to long and very heavy. In the lowest exposure category, about twice as many more deaths from other respiratory or circulatory causes occurred than could normally be expected; and these too can probably be attributed directly or indirectly to their occupation (McDonald *et al.*, 1973). Thus, in all, asbestosis and related cardio-respiratory disease was probably responsible for about 100 (3%) of the 3270 deaths occurring in the cohort. Virtually all these deaths were in men with 800 or more mppcf-years of exposure.

No comparable figures are yet available from the Ballangero survey.

EXPERIENCE IN OTHER COUNTRIES

Information from other chrysotile mining areas is scanty and does little to confirm or to modify the conclusions outlined above. The experience of Russian mine workers is of particular importance but, although Kogan [2] of Sverdlovsk has indicated that his findings are similar to those made in Quebec, this awaits documentation. A report by Kogan & Troitsky (1966) indicates that the main dust hazard was in the mills (concentrating plants), and that, as in Quebec, effective control began in the early 1950's. By 1963 substantial reduction had been achieved, but 95% of measurements still exceeded the permissible Russian limit of 2 mg/m^3, although only 20% were above 10 mg/m^3. By 1962 the prevalence of asbestosis in the mill workers had fallen to about a tenth of the 1950 level, and the disease was of much lower severity; however, no rates were given. It is stated by Kogan *et al.* (1971) that 90 ex-employees from the asbestos industry in the central Urals who died between 1948 and 1967 showed indications of asbestosis; of these 80 had worked in the mills and 10 in the mines. Of the total, 34 died of cancer (8 cases in the respiratory organs), 16 of pulmonary tuberculosis, 14 from circulatory disease, 10 of other well-defined causes, 12 of uncomplicated asbestosis and 4 of unknown causes. Bearing in mind the similarity in output and production methods, the figure of 12 deaths from uncomplicated asbestosis in the Russian industry compares very

[1] Personal communication.

[2] Personal communication.

favourably with the 28 in Quebec (McDonald *et al.*, 1971) during the same period. However, without details concerning the numbers at risk and the exact methods of ascertainment, it is difficult to interpret these findings.

Data from workers in the chrysotile industry in the Shabani-Mashaba region of Rhodesia were presented by Gelfand & Morton (1970). During a 5-year period (1963–67), 39 cases of asbestosis were diagnosed among 8336 employees. All but 2 of the cases occurred in men who had worked for over 8 years in the industry. The permissible dust standard in Rhodesia was 300 particles per cc (about 8 mppcf); and, as elsewhere, the main hazard was found to be in the mills.

The chrysotile industry of South Africa has a work force of about 1350 men; Sluis-Cremer [1] reviewed the X-rays from cases certified as pneumoconiosis during the 12-year period 1960–71 and found 19 cases which he considered to be asbestosis. These findings are closely in line with those of Gelfand & Morton, but comparison with Quebec, Italy or Cyprus does not seem possible.

EXPOSURE-RESPONSE

Safe environmental standards depend on knowledge of exposure-response relationships, and on the concept of what constitutes an acceptable level of risk. A 1% risk of acquiring clinically significant disease during a 50-year working life was used in framing recent British asbestos regulations, and this seems a reasonable point of reference. For chrysotile mining and milling, there are only the data from Quebec, Cyprus and Italy which relate exposure to incidence or prevalence of asbestosis. The clinical significance of minor radiographic changes is uncertain, and probably only irregular small opacities of category 2 and pleural thickening of grade 2 have an appreciable effect on function. In the Quebec and Cyprus surveys, the dust category in which 1% of subjects had this degree of radiographic change was 100–199 mppcf-years. For non-smokers in this category there was a reduction of about 10% in mean value of the more sensitive indices of respiratory function. Assuming that one may extrapolate arithmetically, this suggests that a worker should be exposed to an average concentration of not more than 2 mppcf over a 50-year working life. The fibre equivalent of this exposure level has not been established. Available data suggest that, in Quebec, this may be in the region of 5 fibres per ml. It remains doubtful, however, whether conversion to a fibre equivalent can have much epidemiological validity.

[1] Personal communication.

ADDENDUM

Since presentation of this paper, further environmental measurements in the Quebec mines and mills indicate that the problem of a dust-fibre equivalent is even more complex than anticipated and no single factor is likely to prove satisfactory.

SUMMARY

The results of epidemiological surveys of chrysotile production workers in Quebec, Italy and Cyprus appear to be reasonably compatible. Information from the USSR, Rhodesia and South Africa is not at present comparable, but there is nothing to suggest that experience in these countries is likely to have been very different. The data therefore seems sufficient to justify the conclusion that if the risk of clinically significant disease is to be kept below 1% for a working life of 50 years, workers in this industry should not be exposed to dust concentrations of more than 2 mppcf. Further studies are needed to confirm this estimate and to determine whether there is any valid fibre equivalent.

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Amosite and crocidolite mining and milling as causes of asbestosis

G. K. SLUIS-CREMER [1] & R. S. J. DU TOIT [2]

The findings presented in this paper must be assessed with the following points in mind:

(1) that dust concentrations which caused the observed cases of asbestosis and pleural plaques occurred in the past, but that such heavy concentrations do not exist today;

(2) that the dust concentrations referred to were recorded by means of konimeters and thermal precipitators;

(3) that preliminary observations in another study tend to indicate that the fibre concentration estimated in konimeter and thermal precipitator samples must be multiplied by a factor of 1 to 6 in order to obtain a correlation with fibre concentrations assessed by collection on filter paper. The particular factor to be used is determined by the type of fibre involved; and

(4) that the safe standard arrived at represents a long-term mean; thus the equivalent short-term standard is multiplied by a factor of 1 to 3, depending on the coefficient of variation of the relevant dust level. Hence, if the membrane filter is used the short-term standard may be as high as 45, depending on the type of fibre and the coefficient of variation of the dust level.

MINERALOGY

There are many similarities between amosite and crocidolite asbestos. Amosite is an iron-magnesium silicate and crocidolite a sodium-iron silicate. Both occur in the Transvaal in seams of banded ironstone lying just above a transition zone, which itself lies on the dolomite. The sodium (Na_2O) content of this layer decreases, and the magnesium (MgO) increases as one moves from south to north in North-West Cape Province; thus crocidolite occurs in the south, giving way to amosite in the north. In north-east Transvaal amosite occurs with the crocidolite seams, and in general the Na_2O, or crocidolite content of the ironstone decreases almost to zero, while MgO increases to 7%, when one travels eastward to Penge, where only amosite is found.

The iron (FeO) in amosite may vary from 40% to 31% in the silky montasite, so that there is some chemical variation in the fibres. There are a number of other associated minerals in the banded ironstone, i.e., chert, magnetite, stilphomelane, minnesotaite, riebeckite, grunerite and iron carbonate; and large amounts of carbon and sulphur, the former in the form of graphite, are to be found with amosite.

In the oxidised zone, the magnetite and other iron compounds are transmuted to limonite (griqualandite); grunerite becomes altered to an apple green clay called nontronite [3].

RECOVERY TECHNIQUES [4]

Although in the past a large amount of hand cobbing was done, this method of recovering asbestos has virtually disappeared; however, hand sorting on moving belts is still carried out. In the mill, fibre is extracted by repetitive crushing and air suction; and

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[3] *Asbestos Mining in South Africa*, Shapiro, H. A., ed., in preparation.
[4] *Ibid*

Table 1. Dust concentrations, 1940 to 1971 [a]

Period	Fibre and particles per cm³					
	Crocidolite				Amosite	
	Cape		Transvaal			
	S	U	S	U	S	U
1940–45	2687	1139	–	–	1458	350
1946–50	1742	483	–	–	571	315
1951–55	1036	404	–	–	646	445
1965–66	375	370	633	197	495	130
1968–69	250	192	246	167	522	161
1970–71	273	146	110	96	354	150

S = Surface, U = Underground.
[a] Measured by konimeter up to 1965, thereafter by thermal precipitator for particles and fibres larger than 0.5 micrometre.

the freed fibre is screened to allow rock, grit and dust to drop out. Since this latter process is a dry one there is appreciably more dust in the mill than in the mine [1] (Tables 1 & 2).

Table 2. Quartz in the dust (by X-ray diffractometer)

		95% of confidence limits
NW Cape Crocidolite (S)	7.4%	5.5– 9.3
,, ,, (U)	11.0%	8 –14
N Transvaal Crocidolite (S)	18.7%	8.8–28.6
,, ,, (U)	23.6%	15.9–32.3
Amosite (S)	15.4%	8.5–22.3
,, (U)	10.0%	3.3–16.9

Appreciable amounts of free quartz may thus be present in the ambient dust; and, indeed, occasional silicotic islets may be found at autopsy.

PRODUCTION AND STAFF [2]

In 1970, 131,812 metric tons of Cape Blue, 97,300 tons of amosite and 52,801 tons of chrysotile asbestos were produced. In 1960, the mines used 125 employees per 1000 metric tons produced; by 1970 this number had fallen to 69 employees. In 1970 the complement in all asbestos mines consisted of approximately 1000 Caucasians and people of mixed race and 19,000 Bantu workers.

[1] See p. 138 of this publication.
[2] *Asbestos Mining in South Africa*, Shapiro, H. A., ed., in preparation.

HISTORY OF MEDICAL CONTROL

Shortly after the 1939–45 war, the Department of Mines carried out a radiological survey in the asbestos fields. Asbestosis was found in a large number of those surveyed, many of whom had worked in the asbestos mines or mills for a long time.

Since 1954 all asbestos mines have been controlled under the Pneumoconiosis Act. This requires regular medical and radiological examinations for a Certificate of Fitness, which entitles the holder to work in a dusty atmosphere. The certificate must be renewed annually. In addition, controlled mines are inspected regularly by officials of the Government Mining Engineer's Division of the Department of Mines, and dust control regulations are applied.

SERVICE AND RESIDENCE-TIME IN ASBESTOSIS

Sluis-Cremer (1970) was able to follow up a group of white miners who were employed in crocidolite and amosite mines between 1954 and 1958 (Tables 3 & 4).

Table 3. North-West Cape. Incidence of asbestosis [a]

		0–4.9 years' service		
60(0) [b]	22(0)	35(0)	13(1)	1(0)
0–4.9 [c]	5–9.9	10–14.9	15–19.9	20+
		5–9.9 years' service		
	56(0)	27(0)	10(0)	4(2)
	5–9.9	10–14.9	15–19.9	20+
		10–14.9 years' service		
		67(4)	15(2)	3(1)
		10–14.9	15–19.9	20+
		15–19.9 years' service		
			45(5)	10(0)
			15–19.9	20+
		20+ years' service		
				23(8)
				20+

[a] This table and Table 4 first appeared in *Environmental Research* (1970, vol. 3, p. 310). They are reproduced here by kind permission of the editor of that journal.
[b] The figures in parentheses indicate number of cases of asbestosis in the group.
[c] Years of follow-up.

In northern Transvaal the asbestos mining labour force is found mainly at the amosite mine at Penge. The Transvaal crocidolite mines have a relatively small complement of workers, totalling some 1800 persons.

Table 4. North-East Transvaal. Incidence of asbestosis

		0–4.9 years' service		
11(0)	12(0)	16(0)	10(1)	0(0)
0–4.9	5–9.9	10–14.9	15–19.9	20+
		5–9.9 years' service		
	14(0)	6(0)	9(1)	3(1)
	5–9.9	10–14.9	15–19.9	20+
		10–14.9 years' service		
		19(2)	9(1)	3(1)
		10–14.9	15–19.9	20+
		15–19.9 years' service		
			21(6)	0(0)
			15–19.9	20+
		20+ years' service		
				14(5)
				20+

It can be seen that the incidence of asbestosis and the latent period for its development are very similar in the two groups. The radiological appearances of asbestoses found in the North-West Cape and in north-east Transvaal are indistinguishable.

CALCIFIED PLEURAL PLAQUES

In films from both areas a considerable number of calcified pleural plaques were found, very often as the only sign of asbestos exposure. Of a group of 79 persons certified as asbestosis patients, 41 had calcified pleural plaques as the only evidence of abnormality. A high prevalence of plaques is found both in the North-West Cape and north-east Transvaal. In both of these areas calcified pleural plaques are seen in X-ray films of the population living in the neighbourhood of asbestos mines and mills. Of 1130 persons who had not worked at the asbestos mines of the two areas surveyed, 27 showed the presence of calcified pleural plaques. The prevalence of calcified plaques in these persons is thus about 2.5% (Report, 1964).

Although such plaques occur infrequently in people associated with the chrysotile mines, a survey has not yet been carried out in that area.

MAXIMAL ALLOWABLE DUST CONCENTRATION STANDARD [1]

From the cumulative dust exposure records of the National Research Institute for Occupational Diseases, which were obtained through investigations carried out by the Cumulative Dust Exposure Sub-committee, the authors have attempted to establish a dust concentration standard at which workers probably would not develop asbestosis during a working life-time.

[1] du Toit & Sluis-Cremer, unpublished data.

DATA

Data obtained from the following groups were used for the preliminary estimations. (It was only possible to assess the exposure records of white miners and those of mixed race, as the records of service of the Bantu miners have not yet been investigated. It is planned to do this in the near future.):

(1) Persons with parenchymal asbestosis, with service in amphibole asbestos mines only.

(2) Thirty-seven Caucasian miners with parenchymal asbestosis.

(3) Forty-two persons of mixed race with parenchymal asbestosis.

(4) Forty-one persons with calcified pleural plaques only.

The dust dosage during the working life was estimated for each person from his record of service in which the mine worked on, the type of employment and the length of employment are given. Dust concentration data were available for most mines and mills; and figures for types of employment and the calendar periods of employment were also available from the Air Quality section of the Government Mining Engineer's Division. In the absence of graphs for individual mines, information for mine groups was used; but it was necessary to do this in only an insignificant number of cases. Dust concentration is expressed as actual long fibres/cm^3; actual short fibres/cm^3; and long fibre equivalents/cm^3.

To obtain this last parameter, relative toxicity factors were allocated to the different constituents of the sample as follows:

Fibres shorter than 5 μm	—0.2
Quartz particles	—0.5
Other particles	—0.1
Fibres longer than 5 μm	—1.0

Residence-time estimates were assessed as the period in years between the date when the person

was first exposed to dust and the date of his last X-ray. It was considered that a study incorporating residence time might indicate whether asbestosis is a progressive disease or not.

RESULTS

(1) du Toit (1968) reported that 115 persons exposed to dust in amphibole mines developed asbestosis only after a mean service of 15 years. The mean dust concentration to which these persons were exposed from 1942–1958 was estimated at 420 quartz equivalents/cm^3.

Long asbestos fibres produce a diffuse parenchymal lesion, differing from the nodular lesion seen in silicosis. It is therefore suggested that calculation of the long fibre equivalent should be based on the assumption that long asbestos fibres produce twice as much fibrosis as does quartz.

Disregarding the possibility of progressive asbestosis and assuming a 50-year period for the development of asbestosis, the allowable standard is arrived at as follows: 3.150 ÷ 50 = 60 long fibre equivalents/cm^3. If a 40-year period is taken, the long fibre equivalent would be 79; and for a 30-year period it would be 105.

Depending on the long fibre component of the dust, which for the purpose of this study was taken as 30%, the actual long fibre concentration can be calculated as 19 for 50 years, 29 for 40 years and 53 for 30 years of working life.

If the progression of asbestosis is linear (and this has not been proven), a factor must be used to represent the rate of progression. For the purposes of this study, a factor of 0.5 has been taken as the power to which the dust level must be raised to express change in residence time. In other words, if the exposure time is doubled the dust level should be one quarter of the original. Using a factor of 0.5 and the long fibre equivalents calculated for 50, 40 and 30 years as above, the allowable concentration will be 19, 29 and 53 long fibre equivalents, respectively.

The actual long fibre counts/cm^3 will therefore be 6, 9 and 16, respectively.

(2) These calculations can be applied to the records of thirty-seven Caucasian persons found to have asbestosis (X-ray reading 1/0 and upwards) (Table 5). These were found in a group of workers who were employed in asbestos mines on 30 November 1970. Their mean dust dosage was estimated at 1370 long fibre equivalents/cm^3-years.

Table 5. Dust dosage *versus* proportion with asbestosis. Thirty-seven Caucasian persons

Dosage range (and mid-point) long fibre-equivalents/cm^3-years	Number exposed	Number with asbestosis [a]	Proportion with asbestosis [b]
250 and less (125)	124	2	0.016
251–500 (375)	54	2	0.037
501–1000 (750)	110	8	0.073
1001–2000 (1500)	99	11	0.111
2001–4000 (3000)	41	10	0.244
4000+ (7500)	9	4	0.444

[a] Diagnosis of asbestosis 1/0 and higher.
[b] Not existing prevalence.

At a cumulative dust dosage of 1370 long fibre equivalents/cm^3-years, and disregarding progressiveness, the allowable standard for a 50-year exposure period would be 28 long fibre equivalents/cm^3; for 40 years, 34; and for 30 years 46 long fibre equivalents/cm^3. However, progression should be allowed for. Since the mean residence-time of the dust in the lungs of this group was 13 years, the dust level for a residence-time of 50 years can be estimated at 7 long fibre equivalents/cm^3, or 2 long fibres/cm^3; for 40 years, 10 long fibre equivalents/cm^3, or 3 long fibres/cm^3; and for 30 years, 20 long fibre equivalents/cm^3, or 6 long fibres/cm^3.

(3) In forty-two persons of mixed race who had asbestosis (Table 6), the mean cumulative dust dosage was estimated at 3650 long fibre equivalents/cm^3-years, indicating a much higher number of long fibres/cm^3 in this group for the same working-life periods as the previous group. The difference between this estimate and that of 1370 for Caucasian

Table 6. Dust dosage *versus* proportion with asbestosis. Forty-two mixed-race persons

Dosage range (and mid-point) long fibre-equivalents/cm^3-years	Number exposed	Number with asbestosis	Proportion with asbestosis
0–250 (125)	71	0	0
251–500 (375)	11	0	0
501–1000 (750)	9	1	0.11
1001–2000 (1500)	6	2	0.33
2001–4000 (3000)	5	2	0.4
4000+ (7500)	2	2	1.00

miners is statistically significant (95% confidence limit). The persons of mixed race also had a longer residence-time. Larger dosage combined with longer residence-time suggests that this group on average had more advanced asbestosis than did the thirty-seven Caucasian persons, but no allowance has been made for the different degrees of severity.

There are administrative and social reasons which could explain the difference between the Caucasian and mixed-race groups. Employees who have left the mines often remain for a long time in ignorance of new provisions in the Pneumoconiosis Acts, and therefore years may pass before they present themselves for examination.

(4) Forty-one persons (eight Caucasian and thirty-three of mixed race) with calcified pleural plaques only, had a mean exposure time of 2400 long fibre equivalents/cm^3-years.

In this paper we have suggested assessments which should be made at the present time in South Africa for setting maximum allowable concentrations of asbestos dust. South Africa is not alone in having to set these standards; until the full results of the Cumulative Dust Exposure Project are available we feel that it is premature to lay down any such standard. The project will be carried out with all possible speed.

The Cumulative Dust Exposure Asbestosis Subcommittee consists of Dr R. S. J. du Toit (Chairman), Prof I. Webster, Dr G. K. Sluis-Cremer, Mr R. E. G. Rendall and Mr L. Isserow. The dust measurements were taken by the staff of the Air Quality Research Section of the Government Mining Engineer's Department and the Department of Industrial Hygiene of the National Research Institute for Occupational Diseases.

SUMMARY

A short summary is given of the geology, chemistry and mining and dust conditions in amphibole mines.

The importance of residence-time as well as of dust exposure is stressed.

A maximal allowable dust concentration standard has been assessed from various data. It is believed to lie between 2 and 15 actual long fibres per ml, depending on the degree and mode of progression of exposure, particular working conditions and the composition of the dust.

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Anthophyllite mining and milling as a cause of asbestosis

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Anthophyllite asbestos, commercially produced in Finland since 1918, is known to cause asbestosis (Wegelius, 1947; Noro, 1968). There is also evidence of an excess risk of malignant tumours in workers exposed to the dust of this mineral (Kiviluoto & Meurman, 1970). However, despite a careful search, no mesotheliomas associated with exposure to anthophyllite asbestos have been detected in Finland (*ibid.*).

Finland's only anthophyllite quarry, at Paakkila, annually produces 10,000 to 12,000 tons of asbestos, or 0.3% of world production. Accordingly, the number of workers exposed to the occupational risk is relatively low. The turnover rate of the workers has been considerable, and those employed for a long time have been moved within the company to different jobs. Thus, the intensity of exposure has varied from time to time in many of the workers.

Reliable data from the company's personnel register are available from 1936, when the notification of occupational diseases became compulsory in Finland. Since then, 1676 persons have been registered as workers or other employees at the mine. At the end of 1971 the number employed totalled 121.

In 1952 the Institute of Occupational Health undertook periodical health examinations of the workers at Paakkila, which initially involved only an ordinary chest X-ray. Some biochemical and pulmonary function tests were added later. However, a noticeable number of workers have been referred to the Institute for thorough medical examinations since 1950.

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Information regarding working conditions, particularly of dust concentrations, is not available prior to 1965. However, in four environmental surveys made by the Institute in the mine and the mill between 1965 and 1971, nearly all count concentrations exceeded the threshold limit values (TLV). The level of past dustiness has probably been equally high or even higher.

This paper reports some of our experiences concerning occupational exposure to anthophyllite asbestos from the clinical and epidemiological points of view.

STUDY POPULATION

Of the 1676 persons registered since 1936, 423 had an exposure time shorter than three months. These were excluded from the study population. The remaining 1249, 1044 men and 205 women, entered the study cohort at different times during the follow-up from 1936 to 1972. A noticeable number of farmers from the neighbourhood have been occasionally employed at the company. The longest

Table 1. Distribution of total exposure time, 1936–1972

Exposure time, years	Number of workers
<1	494
1– 5	469
6–10	103
11–15	62
16–20	31
21–25	32
26–30	30
>30	16
Unknown	13
Total	1249

known working time was 36 years. The number of persons exposed for more than 11 years was 170 (14% of the population). The distribution of exposure time is shown in Table 1.

The number of subjects in various phases of the study reported upon here varies according to the aims and design of the study and the availability of the data on exposure, etc., required for the definition of the samples.

Table 2. Distribution of the asbestosis cases according to the duration of exposure until diagnosis

Exposure time, years	Cases of asbestosis	
	Number	Per cent of the cohort with same exposure time
< 1	0	0
1– 5	7	1
6–10	20	19
11–15	23	37
16–20	8	25
21–25	21	65
26–30	13	43
> 30	8	53
Unknown	5	38
All	105	8

CASES OF ASBESTOSIS

There were 105 known cases of asbestosis, 89 men and 16 women, in the study cohort. Classification of the cases by duration of exposure is made in Table 2.

The year of diagnosis, divided into five-year groups, and the number of new cases are presented in Table 3. All known deaths among patients in this group are included and the mean survival times after diagnosis are also shown.

Table 3. Distribution of new cases of asbestosis, number of deaths, and mean survival time after diagnosis in 105 asbestos workers who developed asbestosis

Year of diagnosis	New cases of asbestosis	Number of deaths [a]	Mean survival time after diagnosis, years
1941–45	21	18	8.6
1946–50	19	14	13.0
1951–55	22	13	10.7
1956–60	8	3	9.0
1961–65	9	3	4.2
1966–70	12	3	2.5
1971	14	0	
Total	105	54	

[a] In the old and new cases of asbestosis.

Table 4. Age and sex structure of workers and ex-workers at the company in 1967

Age, years	Employed		Retired		All	Per cent
	Men	Women	Men	Women		
15–24	11	1	–	–	12	9
25–34	19	3	1	–	23	16
35–44	38	2	2	–	42	30
45–54	21	7	4	4	36	26
55–64	10	–	4	–	14	10
65–74	1	–	3	2	6	4
75+	1	–	2	–	3	2
Unknown	2	–	2	–	4	3
Total	103	13	18	6	140	100

THE 1967 STUDY

Five years ago we made a medical investigation of the workers at the company. The sample consisted of 140 workers who were either employed (116) or retired (24). The geographical mobility of the examined persons was low; 70% of them have been living in the neighbouring district for 25 years or more. The age and sex structure of the present sample is given in Table 4. The distribution of exposure time is presented in Table 5.

Table 5. Duration of exposure of workers and ex-workers at the company in 1967

Years of exposure	Number	Per cent
< 2	46	33
3–4	5	4
5–9	18	13
10–14	19	14
15–19	28	20
20–24	14	10
25–29	7	5
≥ 30	3	2
Total	140	100

The investigation included a chest X-ray, a questionnaire on smoking habits, history of respiratory diseases and subjective symptoms for respiratory illness. A battery of lung function tests was also performed.

From the X-ray films, asbestosis was found in 37 (27%) individuals in the sample. Eighteen of them

showed mild, 12 moderate and 7 marked signs of asbestosis. The individuals suffering from asbestosis were older, they had had a longer exposure time, they had been living longer in the locality and had a longer history of smoking, as compared to those who did not have asbestosis. They also complained of significantly more symptoms connected with bronchitis and shortness of breath. This group had lower vital capacity and forced vital capacity values.

Despite the small size of the sample, the results of vital capacity, forced vital capacity and total lung capacity tests in the non-asbestosis group (87 persons) were divided into five sub-groups according to the duration of exposure. No significant differences in the lung functions were found among the sub-groups.

An analysis of the dependence of respiratory symptoms and clinical findings on the duration of exposure showed that, in most cases, prevalence of respiratory disease was not limited to the more exposed; however, statistical significance limits were infrequently reached, probably because of the small numbers of subjects in the various sub-groups of the analysis.

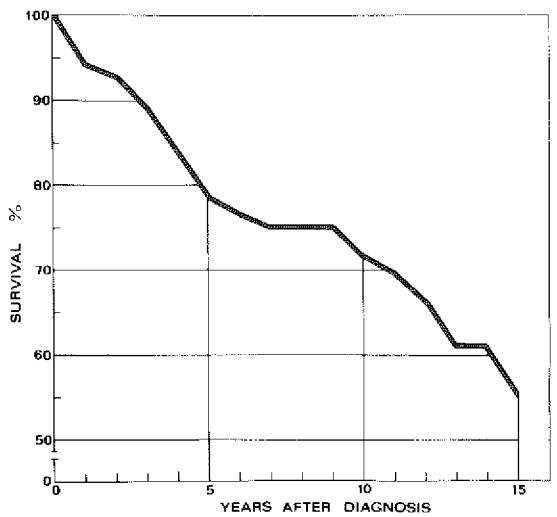

Fig. 1. Survival curve for 56 asbestosis patients diagnosed 1937–52.

MORTALITY

In a study of the mortality of workers employed for at least three months at the plant between 1936 and 1966, the data on the underlying cause of death as recorded on the death certificate showed a notable excess of observed over expected deaths for two major chronic illnesses, i.e., asbestosis and respiratory tuberculosis (Nurminen, 1972). Of all registered 222 deaths, there were 24 for which asbestosis was an underlying cause, and an additional 17 deaths with asbestosis listed along with another underlying cause of death. Thirty-one deaths were observed, compared with the 12 expected, due to tuberculosis of the respiratory system as an underlying cause.

A follow-up study of individuals suffering from asbestosis was made in order to summarise their survival experience (Nurminen, 1972). The study group consisted of all 56 of those patients whose diagnosis was made between 1937 and 1952 and who had filed a claim for insurance benefits with an insurance company. The 5-, 10- and 15-year survival rates were 78.5, 71.3 and 55.4, respectively. In the successive five-year periods after diagnosis of asbestosis, the accumulated survival rate was reduced by 22.7, and 16%. The results are presented in Figure 1.

DISCUSSION AND CONCLUSIONS

Exposure to anthophyllite asbestos dust at the quarry and mill in question causes asbestosis. Its incidence seems to correlate with the duration of exposure, as is the case with other asbestos mineral dusts. Of those workers who had an exposure time of more than 25 years, almost every second person (47%) suffered from asbestosis. Sixty-six per cent of all known cases have had an exposure time of over 11 years. The morbidity figures clearly show the danger of this work when employment has lasted six years or more. Asbestosis has been diagnosed in one-third of the 286 individuals in this exposure group. Such common occurrence of asbestosis is certainly due to high dust concentrations in the air of the plant. Unfortunately, exposure to other contributing factors is hard to measure.

From the clinical point of view, anthophyllite asbestosis has not been studied sufficiently to draw conclusions regarding the different clinical aspects of the disease. This is due to the small size of the group with a long employment time, and furthermore to the fact that the exposed cases are distributed over a period of more than 30 years.

Our data, based on medical examinations of individuals suffering from asbestosis, show that some

dominating features seem to be typical for these cases. Unlike other asbestos minerals in their biological effects on man, anthophyllite dust gives rise to pleural changes at a very early stage of exposure. Later, pleural thickenings and calcifications can be seen in nearly all cases. However, because non-occupational pleural plaques are common among inhabitants in the district (Kiviluoto, 1960; Meurman, 1966), we cannot evaluate those findings alone as diagnostic. In cases of asbestosis, decreased lung functions, particularly lung capacities and the transfer factor can be measured; but there have been no significant changes in the health of workers without asbestosis in relation to the length of exposure.

In view of our systematic surveys of all retired persons in the population under study, we believe that the 105 cases of asbestosis found correspond to the incidence of asbestosis among persons exposed to anthophyllite asbestos dust at the mine and mill. Nevertheless, in a population followed for more than 30 years some cases must be missed. On the other hand, the workers are familiar with the compensation systems for occupational diseases, and they do not fail to take advantage of them. Our collaboration with both the insurance company and with the factory have been extremely good.

The survival curve of asbestos patients presented above (Fig. 1) represents a selected group of workers who received health insurance compensation from the insurance company responsible for all personal insurance at the plant. As the year of diagnosis in these cases belongs to a period from 20 to over 30 years ago, their survival curve may not quite correspond to the actual situation of all registered cases today. One can argue that the diagnostic technique permits us to discover new cases at an earlier stage than in the past. Furthermore, the concomitant diseases, such as respiratory tuberculosis, have become somewhat less severe than before; and the medical and socio-medical care of patients suffering from asbestosis have markedly improved in the last two decades.

SUMMARY

Clinical and epidemiological data concerning occupational exposure to anthophyllite asbestos are reported. The data base is a cohort of 1249 individuals with exposure at an anthophyllite mine and mill since 1963, 105 of whom are known cases of asbestosis. Results and the progress of different phases of the project are illustrated and discussed. A picture of a dangerous working environment arises, with heavy health risks, especially for workers with long-lasting exposures.

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Asbestosis in textile manufacturing

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Asbestos textiles constitute a minor part of the world's textile industry and the textile uses of asbestos consume only a minor part of the world's production of asbestos. However, it is only in recent years that cases of asbestosis in the other asbestos applications in the United Kingdom have exceeded those in the textile industry.

In the UK the asbestos textile industry consumes over 10,000 tons of chrysotile annually, weaves about 4 million yards of cloth and spins about 10 million miles of yarn. The total asbestos imports into the UK are 144,000 tons of chrysotile and 20,000 tons of amosite, but no crocidolite since 1970.

In asbestos textile mills chrysotile group 3 is the main source of asbestos yarn, although crocidolite can also be used for this purpose. There is experimental evidence to suggest that chrysotile is less able to penetrate the depths of the lungs than is crocidolite (Timbrell, 1969), and also that it is less fibrogenic than amosite or crocidolite (Wagner & Skidmore, 1965). In the UK crocidolite is no longer used in the manufacture of asbestos textiles.

Most countries do not provide official statistics; however, it is known that the largest asbestos yarn producers are the UK and the USA; it is estimated that their combined output is about the same as that of all the other non-communist countries put together. Germany, Japan and France produce about 40% as much as do the UK and USA, while Italy and Canada produce about 10% of this amount. These seven countries represent some 85% of the total world production of asbestos yarn. The remaining 15% is made up by the production of the following countries: Argentina, Austria, Belgium, Brazil, India, Israel, Mexico, Portugal, Spain, Turkey, Yugoslavia and Venezuela.

There have been two main trends in the uses of asbestos: one, as a fibrous reinforcement of paper or boards fabricated by the use of wet processes; and the other, its use in the textile industry. The use of textile materials reinforced with asbestos preceded its use in wet processes. In the past, asbestos textile production was basically a dry process, whereas asbestos-reinforced products were made basically by wet methods; thus, proportionately more textile workers were exposed to dust than were the workers in the board and paper industry. Merewether & Price (1930) restricted their enquiry to workers in processes "in which there is exposure to pure asbestos or asbestos mixed with a very small proportion of cotton or other vegetable fibre". At that time they calculated that there were 2200 workers involved, of whom they examined 16.5%. The Asbestos Industry Regulations, 1931 placed all textile processes into "scheduled areas", requiring particular supervision, but included in that group comparatively few processes of the reinforcement industry. For example, in a Barking factory making asbestos-reinforced insulation materials (Smither, 1964), only 6% of the workers were in officially scheduled areas. We now know that others were significantly at risk.

Table 1. Comparison of fibre and dust amounts [a]

	Amount of fibre in product (%)	Amount of fibre in dust	
		Total dust (%)	Respirable portion (%)
Textiles	75–85	68	64
Friction materials	30–60	22	36
Pipes	10–30	12	18
Shingle	10–30	25	28
Insulation materials	5–15	4	6

[a] Reproduced, by permission, from Lynch *et al.* (1970).

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Table 2. Dust levels in various operations in textile processes

Reference	Fibre preparation	Carding	Spinning	Plaiting or twisting	Weaving
Lynch & Ayer (1966) USA (9 textile plants) Membrane filter fibres/ml > 5 μm	7.6	7.0	6.2	6.7	3.6
Knox *et al.* (1968) UK (one factory, yearly means) Membrane filter fibres/ml of 5–100 μm	5	$\overline{5}$	$\overline{5}$	4	$\overline{2.5}$
Medek (1969) Germany (one factory) mg/m³ converted as by Schutz (0.24 mg/m³ = 4 fibres/ml > 5 (μm)			6.5	2.4	3.2
Nakamura (1967) Japan (one factory) Calculated as fibres/ml > 5 μm	47.2	8.2			25.3

There are essential differences in the amounts of asbestos in various products and in the amounts of dust produced during their manufacture. Lynch *et al.* (1970) clearly show that textiles contain a higher proportion of asbestos in the product, in the total dust produced, and in the respirable fraction of that dust than do the other products in their study (Table 1). Schutz (1970), comparing asbestos textile manufacture with asbestos cement manufacture in Germany, commented: "In other words, in the asbestos cement industry the respirable fine-dust component amounts to about 15–25% and in the asbestos textile industry to about 35–50%."

Figures extracted from the reports of Lynch & Ayer (1966), Knox *et al.* (1968), Medek (1969) and Nakamura (1967) show similar differences (Table 2). Medek's dust concentrations were originally given in volumes expressed as mg/m³, and these have been modified according to the calculations of Schutz (1970), who demonstrated that 0.24 mg/m³ was equivalent to 4 fibres > 5 μm per ml. This was done in order to make comparison easier. The variations from one operation to another do not appear to be great, except in the Nakamura figures; aside from these, there is general agreement with the observation of Ghezzi *et al.* (1971) that the dust concentrations are greatest during the carding operation.

McVittie (1965) reviewed 247 new cases of asbestosis diagnosed by four of the pneumoconiosis panels, which accounted for 63% of all asbestosis cases diagnosed between 1955 and 1963. Grouping them by occupations he found that 42% occurred in the section of the industry concerned with applying asbestos insulation. McGowan [1] reveals that between 1963 and 1969, the number of cases diagnosed by the same four panels had risen to 471, and that the proportion of these who were insulators had risen to 50%. In 1955–1963, weaving accounted for 7% of cases, while between 1963–1969 it accounted for 4%, although the number of cases remained about the same. The proportion of new cases in carding and spinning had not significantly changed. In spite of technical improvements, these remain the dustiest processes in the manufacture of asbestos yarn by conventional means (Table 3).

[1] Personal communication.

Table 3. New cases of asbestosis diagnosed in 1955–1969. Analysed by principal occupation

Principal occupation	No. of cases 1955–1963	No. of cases 1963–1969	Total
Opening and disintegrating	41 (17%)	45 (10%)	86 (12%)
Insulating:			
Laggers	72 (29%)	134 (28%)	206 (29%)
Sprayers	13 (6%)	15 (3%)	28 (4%)
Mattress makers	5 (2%) } 42%	21 (4%) } 50%	26 (4%) } 49%
Others	12 (5%)	71 (15%)	83 (12%)
Weaving	16 (7%)	14 (3%)	30 (4%)
Carding, spinning, etc.	37 (13%)	51 (11%)	88 (12%)
Slab and pipe making	20 (8%)	19 (4%)	39 (5%)
Brake lining	4 (2%)	10 (3%)	14 (2%)
Miscellaneous	27 (11%)	91 (19%)	118 (16%)
	247 (100%)	471 (100%)	718 (100%)

Table 4. Cases of asbestosis—1948–1968 (for UK and for a Rochdale asbestos textiles factory)

Year	UK asbestosis cases, diagnosed by PMP	Estimated "at risk" population—UK	Asbestosis cases, Rochdale factory	Asbestosis cases in Rochdale employees joined since 1948	Estimated "at risk" pop. in scheduled areas—Rochdale
1948	*	14,445	–	–	1465
1949	*	–	4	–	–
1950	*	–	–	–	–
1951	15	–	1	–	–
1952	15	–	–	–	–
1953	23	–	–	–	–
1954	31	16,500	2	–	1082
1955	48	–	4	–	–
1956	31	–	8	–	–
1957	56	–	–	–	–
1958	27	18,700	2	–	1068
1959	37	–	3	–	–
1960	29	–	1	–	–
1961	43	–	–	–	–
1962	52	–	1	1	–
1963	67	19,600	4	–	908
1964	84	–	4	1	–
1965	82	–	5	–	–
1966	114	–	4	3	–
1967	168	–	8	2	–
1968	130	–	2	1	837
1969	134	–	4	–	–
1970	153	–	8	2	913

* Figures not available.

Italian studies (Vigliani, 1969) have shown that of 586 new cases of asbestosis diagnosed between 1964 and 1966, 57 (9.7%) were in textile or friction material workers. In contrast to the UK findings, 342 cases (58%) occurred in the asbestos-cement industry and only 22 (3.8%) in the building and insulation industry. Textiles in Italy are made of "mixed" types of asbestos, and dust concentrations range from 0.4 to 665 fibres per ml (Ghezzi *et al.*, 1971); however, these authors do not analyse the dust counts according to the textile process involved.

The Digest of Pneumoconiosis Statistics (1970), shows that in 1970 153 new cases of asbestosis were diagnosed in the UK. In a Rochdale asbestos textile factory employing just under 1000 persons there were eight new cases diagnosed. Of these, four had commenced employment prior to 1933 when the Asbestos Industry Regulations, 1931 took effect. Two of the eight had commenced employment since 1948 when the National Insurance (Industrial Injuries) Act, 1946 came into operation; both of these cases are from the opening, carding and spinning processes. The average time for development is 13.1 years with a range from 7–18 years (Table 4).

Lewinsohn (1972) has shown that in the Rochdale textile factory, in which he works, the incidence of asbestosis in persons employed for 20 years or more since 1933 has been reduced from 81% in 1930 to 2.3% in 1968. He has also shown that the number of persons who remained in employment for 20 years or more was only a small proportion of the work force at any one time.

It is now possible to compare yearly mean dust levels at various textile processes in Rochdale in the years 1961, 1966 and 1971. A comparison between personal sampling and static sampling indicates that the former technique better illustrates the actual exposure of the worker. It can be seen that carding and spinning operations still give the highest counts (Table 5a).

For many years, processes subsequent to carding have incorporated damping techniques to reduce dust levels, and these have been very successful; also, dust-suppressed types of asbestos cloth have been available for many years. The early 1970's witnessed the advent of a revolutionary wet dispersion process known by the trade name of "Fortex". This has been developed in the UK under licence from the German inventors, Rex Asbestwerke.

The Fortex process is an entirely new method of producing chrysotile asbestos yarn, in that it involves a chemical process which eliminates the need for

Table 5a. Dust levels—Rochdale asbestos textile factory—1961, 1966, 1971

Department	Process	Yearly mean dust levels (fibres/ml)			
		Long-running thermal precipitator and membrane filter		Static sampler	Personal sampler
		1961	1966	1971	1971
Fiberising	Bag slitting	4.5	4	3	1
	Mechanical bagging	4.0	4.5	4	1
Carding	Fine cards	5.5	5.5	3.5	2
	Medium cards	7.5	8	4.5	3.5
	Coarse cards	7.0	7.5	8	6
	Electrical sliver cards	5.0	2	1.5	1
Spinning	Fine spinning	4	3.5	2.5	3
	Roving frames	5.5	5.5	6	3
	Intermediate frames	5.5	5.5	5.5	3
Weaving	Beaming	8	3.5	0.5	0.5
	Pirn weaving	3	2.9	1.5	1
	Cloth weaving	3	2	2	1
	Listing weaving	2	1	0.5	0.5
Plaiting	Medium plaiting	4	4	4	2

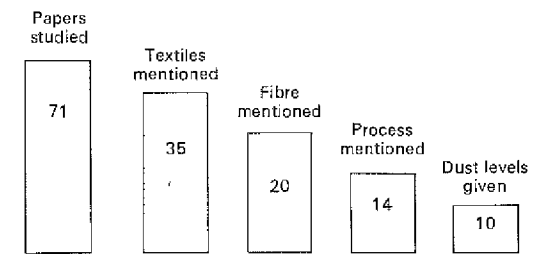
mechanical opening and carding. The asbestos is separated and dispersed by means of chemical reagents into far finer fibrils than has previously been possible. These ultra-fine fibres are then extruded in the form of a film, which is spun into yarn and treated to make it ready for further processing into cloth. Fortex products are remarkable for their cleanliness, the surface of the fabric is very smooth and regular; dust counts obtained in the manufacture of this product are much lower than in the conventional processes (Table 5b).

Table 5b. Dust levels in Fortex processing—1971

	Fine		Medium	
	Static sampler (fibres/ml)	Personal sampler (fibres/ml)	Static sampler (fibres/ml)	Personal sampler (fibres/ml)
Forming	0.5	0.5	0.5	0.5
Spinning	0.5	0.5	0.5	1
Winding	0.5	0.5	0.5	1

Because of the long time-lag between first exposure and the development of the first signs of asbestosis, we will be faced for many years to come with the results of past exposures. It is therefore regrettable that so many reports on the biological effects of asbestos in industry are lacking in exposure measurements. Of 71 papers reviewed among those published since 1964 (Table 6), the majority refer only to "asbestos workers", without mentioning which of the major branches of the industry are involved. Textile workers were mentioned in 35 reports; the type of fibre, which in the textile industry is mainly chrysotile, was specified in 20; the particular textile operation was recorded in 14; but only 10 reported the dust levels to which the workers were exposed.

Table 6. Summary of papers published 1964 to 1970



Enterline & Kendrick (1967) comment, "Unfortunately, historical data regarding dust levels proved to be inadequate. Exposures varied considerably within the plants. The general impression gained in surveying some of these plants is that asbestos dust is less in building product plants than in friction materials plants, and both of these are considerably less dusty than asbestos textile plants." Comparing numbers of deaths in which asbestosis is mentioned in various branches of the asbestos industry, they report the highest death rate in the textile industry (Table 7).

Table 7. Deaths in white males, aged 15 to 64, who worked in selected US asbestos plants at some time during 1948–1952 [a]

By deaths at aged under 65 with asbestosis mentioned, as of 30 June 1963

	No. of workers	Deaths with asbestosis mentioned	Deaths per 1000 workers
Asbestos building products	12,402	10	0.81
Asbestos friction materials	7,510	29	3.86
Asbestos textile products	1,843	24	13.02

[a] Reproduced, by permission, from Enterline & Kendrick (1967).

Lieben (1966), in a study of one factory where both textile and friction materials were produced, found that out of 21 deaths from asbestosis and malignancy, more occurred in textile than in friction material departments. The fibres used were mainly chrysotile, only one card of amosite having been used. The number of men employed in each department is not specified, nor is the total population at risk recorded.

The causes of death in asbestos textile workers with asbestosis do not appear to vary widely from the causes of death reported in other branches of the asbestos industry. O'Donnell *et al.* (1966) report the causes of death in 55 asbestos textile workers who had pathologically-proven asbestosis. There were 28 malignant neoplasms, 23 bronchogenic carcinomas and 5 mesotheliomas of peritoneum or pleura. The authors state that the fibre used was "chrysotile almost exclusively", which does not enlighten those who query the role of possible small amounts of crocidolite or amosite in mesothelioma formation. Lewinsohn (1972) reports 15 cases of mesothelioma in a textile factory, of which the majority were exposed before 1930, and only four experienced their first exposure between 1937 to 1943. He notes the reduction in recent incidence of carcinoma of the lung to expected levels as the incidence of asbestosis has fallen.

However, Avril & Champeix (1969) report that in their experience asbestosis is observed mainly in asbestos textile plants in which the fibre used is 99% chrysotile and 1% crocidolite. They found no cases of carcinoma in one plant, but five in another; they began a study of mesothelioma and asbestos exposure following the finding of seven "random" cases associated with exposure to asbestos.

CONCLUSIONS

There is insufficient data regarding type of fibre, dose, duration of exposure and industrial process in the world literature reviewed to enable an accurate appraisal of dose-response relationships for cases of asbestosis in textile factories in manufacturing countries. Where dust counts have been related to the incidence of disease, it can be shown that asbestosis declines with the practice of strict dust controls. Although some cases of asbestosis still appear in the asbestos textile industry as a result of earlier conditions, evidence suggests that, as the effect of modern techniques of dust control becomes apparent, even fewer cases should occur. In addition, the development of new manufacturing processes for asbestos yarn should be considered as an advance in dust control.

The position with regard to mesothelioma in the asbestos textile industry is not entirely clear. Past use of crocidolite in the UK may be contributing to the number of cases of mesothelioma currently appearing in the textile industry in that country.

There is an urgent need for standardisation of the way in which information is recorded with regard to exposure, in terms of type of fibre, dust concentration, industrial process and duration. Duration of exposure may be more accurately assessed by a more extensive use of personal sampling devices.

SUMMARY

The processes involved in asbestos textile manufacture are briefly discussed with regard to exposure levels. The inadequacy of current information is noted. In the textile industry, where mainly chrysotile is used, it appears that practicable methods of dust control have successfully reduced the incidence of asbestosis.

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Asbestosis in the manufacture of insulating materials

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Studies of the effects of asbestos on the health of those who manufacture and use insulating materials have provided much of the evidence to support stricter controls of asbestos (Leathart & Sanderson, 1963; Selikoff *et al.*, 1965). For a number of reasons, however, some of which will be discussed in more detail later, epidemiological studies in the insulating industry have not provided much precise information as to dose and duration of exposure with reference to particular types of fibre.

USE OF ASBESTOS IN INSULATION

Asbestos has been used commercially as a thermal insulating material for over a hundred years, the first materials used having been mixtures of fibres and sodium silicate.

In a current listing in the USA of 120 types of thermal insulation materials (Malloy, 1969) 20 are shown to contain asbestos, including components of rigid and semi-rigid blocks, sheets, pipe covering, flexible rolls, blankets, felts and batts, cements and sprayable mixes. The major product used in rigid and semi-rigid applications for many years was the so-called "Magnesia", developed about 1885, consisting of 85% magnesium carbonate and approximately 15% asbestos. This material is unsuitable for use at temperatures above 550–600°F (289–317°C) since it is liable to calcine, and other more resistant formulations have been developed to meet the demands of higher-temperature systems. Calcium silicate and asbestos products suitable for temperatures up to 1200°F (650°C) were developed in the 1940's, either as hydrous calcium silicate or high temperature calcium silicate. For even higher temperatures, materials containing diatomaceous earth and asbestos products have been used. In the United States during the 1930's both chrysotile and amosite were used; however, amosite became more widely used until by 1950 it was predominant. The trend toward chrysotile began about 1960.

The quantity of asbestos used in insulation materials in the US has remained relatively constant over the past forty years, i.e., 16,500 short tons used in 1930 *versus* 16,100 short tons used in 1965 (Hesse [3]). This has been so despite a great increase in the total production of insulation materials, and it reflects both an increasing use of fibrous glass and the decreasing percentages of asbestos in older materials. Magnesia, which contains 14–15% asbestos, is now little used; calcium silicate insulation which formerly contained 11–12% amosite, in 1965 contained 7% chrysotile. There have also been changes in the composition of blankets and batts. Cements, which were once 100% chrysotile now usually contain 15% or less chrysotile.

These facts have been presented to make the point that it has been impossible in the insulation industry to cl· ify exposures by type or percentage of asbestos, nor by the presence or absence of other materials such as magnesium carbonate, calcium silicate, and diatomaceous earth. It has been suggested that these might alter or modify the pathologic response (Merewether, 1933) but this has not been adequately investigated either experimentally or epidemiologically.

INCIDENCE OF ASBESTOSIS

There is no agreement on criteria for diagnosing asbestosis, so comparisons from one epidemiological

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or descriptive study to another are difficult; the evaluations of the clinician, the epidemiologist and the attorney are quite different. In this review, we will be forced, for the most part, to use each investigator's own criteria.

One index is mortality attributed to asbestosis, cor pulmonale or chronic respiratory disease. There are many reports which show increased mortality in insulation workers. Selikoff *et al.* (1964) reported 12 asbestosis deaths in 632 insulation workers between 1943 and 1962; successive follow-ups of this group have confirmed the risk. Cooper & Balzer (1968) reported 4 deaths from pulmonary fibrosis and cor pulmonale among 49 deaths that occurred between 1946 and 1965 in insulation workers with over 20 years in the trade. A more recent finding by Cooper & Gaffey [1] shows the standardised mortality ratio for respiratory diseases to be 571.4 for insulating workers in San Francisco who began employment before 1946 (deaths until 31 December 1971). Enterline [2] has found a standardised mortality ratio for respiratory disease of 375.7 in production workers manufacturing asbestos insulation products; there were 5 deaths attributed to asbestosis out of a total of 85 deaths for all causes. Selikoff (1971) found similar mortality patterns in a study limited to a plant manufacturing only amosite insulation products.

Another index of asbestosis is in cases certified for compensation. McVittie (1965) reported that 41% of the newly-certified cases of asbestosis during the period 1955–63 in the United Kingdom came from the insulation industry. By 1970 the proportion had passed 50%. Leathart & Sanderson (1963) pointed out that in 1960 and 1961, 32 of 67 cases certified for asbestosis in the UK were men employed as insulators or asbestos sprayers. Marr (1964) reported 5 cases of disabling asbestosis among insulators in a California naval shipyard. Ahlman & Siltanen (1971) described 56 diagnosed cases of asbestosis in insulators in Finland in the years 1939–1968.

There have been a number of cross-sectional studies providing data on the prevalence of asbestosis which confirm the foregoing. Selikoff (1965) reported abnormal chest roentgenograms in 1117 insulation workers in New York City. Those classified by the author as grade 2 changes occurred only in men with 10 or more years since onset of exposure; grade 3 changes occurred only after 20 or more years. Cooper & Balzer (1968) found definite asbestotic changes in 24% of 386 insulators in San Francisco; in those with over 20 years in the trade 41% had definite roentgenographic changes.

Harries *et al.* (1972) reported a high prevalence of radiological abnormalities both in shipyard laggers and in shipyard workers only indirectly exposed to insulation materials. In a series of studies in New England shipyards Murphy *et al.* (1971) report similar findings. In 1967–70, in a study of 2219 asbestos-exposed workers in the Netherlands (Miedema [3]), including 712 insulating workers, 7.7% were diagnosed as having asbestosis on the basis of chest films, function tests and histories of exposure. This was a larger proportion than was found in workers manufacturing asbestos cement, asbestos floor tiles, or engaged in shipyard work. No quantitative data concerning exposures were obtained, but it is known that chrysotile, amosite and crocidolite had been used.

Studies of pulmonary pathological changes have confirmed these findings. Selikoff (1965) found fibrosis in all of 45 insulation workers who came to autopsy; 44 of these had been in the trade for 15 years or more. He reported that the average severity found in insulation workers was less than that in 11 persons who had worked in plants manufacturing insulation materials.

Pleural calcification is common in insulation workers. Frost *et al.* (1956) found 11 instances of calcified pleura in 31 insulation workers engaged in the trade for 20 years or more. There have been similar reports by Selikoff (1965), Cooper & Balzer (1968), and Harries *et al.* (1972).

The foregoing summarises facts that are well known which confirm that amosite, chrysotile and crocidolite are capable of causing pneumoconiosis. It does not answer questions as to the relative pathogenicity of types, nor the relationships between dose, duration of exposure, and severity of disease. Quantitative information is not lacking (Fleischer *et al.*, 1946; Ferris *et al.*, 1971; Cooper & Balzer, 1968; Leathart & Sanderson, 1963; Murphy *et al.*, 1971) but most of that from the insulating trade is poorly adapted to epidemiological interpretation, and relatively little information has been obtained from the insulation manufacturing industry. The insula-

[1] Unpublished data.
[2] Personal communication.
[3] Unpublished data.

tor's work is characterised not only by the use of a variety of materials, which have changed over the past 30 years, but also by many tasks which range in dust exposures from intense to very slight. Peak concentrations may be high for brief periods, while the time-weighted averages are often deceptively low. While one would expect studies in the manufacture of insulation materials to be more productive, so far none has been conclusive in quantitative terms.

The National Institute of Occupational Safety and Health (1972) in a publication concerning criteria for occupational exposure to asbestos included data on dust concentrations in insulation manufacturing plants, but these were not correlated with effects. Some plants were found to have alarmingly high dust concentrations, mean dust counts of 16–75 fibres/ml, with many counts much higher; others, for similar operations, had dust concentrations below 1 fibre/ml. It is probable that these divergences reflect not only different industrial hygiene practices, but also the differing characteristics of the products being manufactured. Controlled tests of different types of insulation material, in which the actual operations involved in sawing, pounding and tear-out were carried out by skilled insulators in a test chamber, showed significant differences in the dust-producing potentials of different products (Balzer *et al.*, 1972).

Such differences may be reflected not only in total particulate and fibre concentrations, but also in the correspondence between dust counts made by impinger and fibre counts taken from membrane filters. This increases one's reluctance to extrapolate from total dust counts made in the past to fibre counts made by currently recommended methods. While Ayer *et al.* (1965) found that in textile plants 1 million particles per cubic foot (mppcf) corresponded roughly to 6 fibres/ml (greater than 5 μm in length), Cooper & Balzer (1968) reported that no dependable ratio between the two methods could be found in the insulation trade. They found ratios corresponding to 1 mppcf that ranged from 0.2 to over 20 fibres/ml. Other studies in insulation plants have reported areas in which midget impinger counts of under 5 mppcf were associated with fibre counts of over 300 fibres/ml. Thus in many areas, e.g., in lagging operations, there exist airborne fibre concentrations which allow little opportunity for discrimination between types and durations of exposure. This is exemplified by studies in Finland (Ahlman & Siltanen, 1971) where fibre concentrations in insulation plants were found to range from 6 to 122 fibres/ml, and in lagging operations from 34 to 92 fibres/ml.

In brief, the difficulties in defining type, quantity and duration of exposure over periods of 10, 20 or more years have made the insulating trade and the manufacture of insulation materials unfavourable sources of precise information on how these factors affect the development of asbestosis.

SUMMARY

Studies of workers involved in the application of insulation have been consistent in showing a high rate of occurrence for asbestosis in persons exposed to insulating materials containing chrysotile, amosite, or crocidolite. Values for the average dust concentrations that are associated with the production of disease are not reliable because of changing products and alternations between brief periods of high exposure (in sawing, mixing muds, tear-out) and periods of relatively low exposure. These uncertainties make comparisons among different types of asbestos and products impossible.

Studies of workers involved in the manufacture of insulation materials are more likely to reveal significant relationships between dose and response, but there is no published information which permits the evaluation of relative hazards in terms of asbestosis.

Consideration should be given, in both epidemiological studies and studies in animals, to the pathogenic contribution of the materials which accompany asbestos in insulation products, such as magnesium carbonate, calcium silicate and diatomaceous earth, which may modify the fibrotic response.

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Asbestosis in asbestos cement workers

P. E. ENTERLINE [1] & H. WEILL [2]

The bulk of the current world output of asbestos is consumed in the production of asbestos cement and asbestos cement products. No other single use of asbestos has grown so rapidly in recent years. Both chrysotile and crocidolite asbestos are used, the former where flexibility in the finished product is of primary importance, and the latter where strength and resistance to acid corrosion are required.

Exposure of workers occurs during the unpacking and mixing of asbestos fibre, and again when the dried product undergoes cutting, punching and grinding, at which time there is also exposure to silica. Dust exposure is minimal during intermediate stages of the process, when the product is wet.

There are a number of reports concerning pneumoconiosis among asbestos cement workers. El-Sewefy (1969) reported on the chest X-ray examinations of 347 unskilled labourers in a cement asbestos pipe factory in Egypt. These workers were mobile, being exposed to asbestos dust on one shift and to silica and/or cement dust on another. Dust concentrations in the factory were high, with hourly suspended dust counts ranging up to 640 mg/m^3. The proportion with positive chest X-rays increased with duration of exposure, ranging from 50% for workers with under 10 years' exposure to 81% for workers with 20 years' or more exposure. Radiological appearances were not all consistent with asbestosis and often showed mixed dust pneumoconiosis and silicosis.

A similar study of 49 workers in an asbestos cement factory in India, with airborne dust concentrations several times the maximum allowable concentration of 5 million particles per cubic foot (mppcf) of air, showed 27% to have positive chest X-ray findings (Banerji, 1968). Ninety per cent of the workers had been continuously exposed to asbestos dust for five years or more. Twenty-eight per cent of the workers showed vital capacities of less than 74% of the standard value for Indian males, while 41% complained of dyspnoea. Four cases showed some tendency to clubbing, and there was cyanosis in one case.

One of us (HW) has completed the first phase of an investigation of health effects related to dust exposure in the manufacture of asbestos cement products in the United States (Weill *et al.*, 1973). Clinical information was obtained from 908 currently employed, recently retired or separated workers from two plants by means of a personal interview and pulmonary function studies, including measurement of lung volumes and its components, maximum respiratory flow rates, pulmonary diffusing capacity and gas exchange at two submaximal levels of exercise. Chest X-ray films were read according to the UICC/Cincinnati classification. Each worker's job history was obtained from plant records; and exposure assessments were based on dust measurements made over the past 20 years by governmental or company industrial hygienists. Indices for total dust and its constituents, including chrysotile, crocidolite and silica contents were calculated for each job each year and ultimately derived cumulatively for each worker. Initial analysis of data has been concerned with the correlation of dust exposure, pulmonary function and radiographic patterns.

It was found that the mixed dust exposure to asbestos and silica produced both small rounded and irregular opacities radiographically. Table 1 shows that radiographic changes of 2/1 category or above begin to appear after a total cumulative dust exposure of 1000 mppcf-months, with a steady increase

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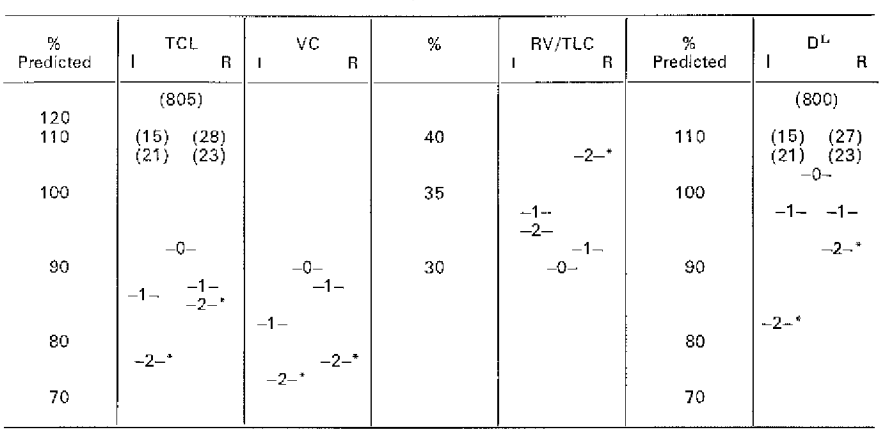
Table 1. Rates (%) of rounded or irregular small opacities for total dust categories

Maximum profusion (UICC/Cincinnatti classification)	Total dust exposure (million particles per cubic foot-months)					
	≤250	251–500	501–1000	1001–2000	2001–4000	4001+
0/0 and 0/–	95	93	94	81	70	60
0/1	4	6	4	11	16	17
1/0, 1/1 and 1/2	0	0	2	3	6	13
2/1+	1	1	0	5	8	10
	(161)	(67)	(105)	(122)	(209)	(231)

in the rates of abnormal radiographic patterns as exposure becomes higher. There was no clear association between silica exposure and rounded opacities or asbestos exposure and irregular opacities, probably because a limited range of exposures to these two materials was encountered in the plants. Profusion of rounded opacities was better correlated with total dust exposure than was profusion of irregular opacities, and cumulative dust exposure was better correlated with the presence of diffuse changes on the chest X-ray than was number of years in the industry.

In order to determine whether the pulmonary physiologic pattern encountered in individuals with well-established irregular opacities (2/1+) differs from that in those with rounded opacities (2/1+), mean values for lung volumes, gas transfer and expiratory flow rates were expressed in per cent of predicted, and these were compared in the two groups. Figure 1 shows that in those persons with either type of small opacity pattern, total lung capacity and vital capacity are significantly lower than in the group without radiographic evidence of diffuse pulmonary infiltrations; and mean values for both of these volumes are considerably lower in the group with irregular opacities than in the group with rounded change. The mean residual volume:total lung capacity ratio was significantly higher in the rounded opacity group than in the negative X-ray group, and also higher than in those with irregular opacities. Pulmonary diffusing capacity was significantly lower with both X-ray patterns than when these changes were absent, being lowest in the group with irregular opacities. The change in diffusing capacity was related primarily to reduction in alveolar volume rather than in diffusion constant. Mean maximum expiratory flow rates were significantly lower in those with definite small opacities than in those without parenchymal change, but the type of opacity could not be distinguished on the basis of

Fig. 1. Pulmonary function mean values in groups with small irregular and rounded opacities[a]



[a] * '2' is significantly different from '0' at 0.01. '0', '1' and '2' represent profusion of opacities: 0 = 0/–, 0/0; 1 = 1/0, 1/1, 1/2; 2 = 2/1+. 'I' and 'R' represent type of small opacities: I = irregular only; R = rounded only.

this measurement. When pleural changes were noted radiographically, lung volumes and flow rates were affected adversely in the presence of both types of opacities, while diffusing capacity was further reduced only in those with rounded change.

It appears that individuals with a moderately advanced profusion of irregular opacities tend to have lower lung volumes and pulmonary diffusing capacity than those with rounded opacities. Hyperinflation is associated with the rounded nodular change, while measurements of flow rates do not distinguish the type of opacities. These physiological patterns are consistent with the hypothesis that the small rounded opacities are primarily the effect of silica exposure and that irregular opacities indicate asbestos effect.

Some studies have been made which permit comparisons of the occurrence of asbestosis in asbestos cement workers with that in other workers in the asbestos products industry. Bohlig (1970) studied workmen's compensation cases for asbestosis and asbestosis with lung cancer in West Germany for the years 1958–66. He concluded that the incidence of asbestosis was lower in asbestos cement workers than in asbestos textile workers. For asbestosis, the ratio of cases from the asbestos cement industry to those from the asbestos textile industry was estimated at 1:11; while for asbestosis with lung cancer it was 1:5.

Somewhat similar findings had been reported earlier by Enterline & Kendrick (1967). In a study of mortality at ages 15-64 in a cohort of 21,755 men working in the American asbestos products industry, they found that the asbestosis mortality rate was very much lower in the building products industry than in asbestos friction materials and asbestos textile plants. The contrast was not so striking for respiratory cancer, however, and after adjustment for age-time differences, respiratory cancer mortality rates were slightly higher for building products workers than for friction materials workers.

Vigliani (1969) has reported on new cases of asbestosis compensated in Italy during 1964–66, giving the occupations in which the subjects were involved. Of a total of 586 cases compensated, 342 were workers making cement-asbestos and refractory material. Unfortunately, the populations at risk are not given, so that the risk in workers exposed to asbestos cement relative to other products cannot be assessed.

Currently, one of us (PE) is conducting a study of the mortality experience of ages 65 and over of a retired population of asbestos workers in the United States. These are workers who spent most of their working lifetime mining or milling asbestos or as production or maintenance employees in the manufacture of asbestos cement products, insulation materials, packing and gaskets, friction materials, textiles, paper and flooring. For each worker, data compiled include a time-weighted measure of total asbestos dust exposure, type of work performed and type of asbestos used. For 1348 workers retired during the period 1941–67 on reaching age 65 and followed for deaths until 1969, a linear relationship was found between the time-weighted measure of asbestos dust exposure (as measured by the midget impinger) and deaths from asbestosis, with annual mortality rates for asbestosis ranging from 35 per 100,000 for men with exposures of less than 125 mppcf-years to 1139 deaths per 100,000 for men with exposures of 750 mppcf-years or more. Asbestos dust exposure was also found to be highly related to respiratory cancer mortality, with the excess

Table 2. Observed and expected deaths and standardised mortality ratios (SMR'S) for selected causes of death by type of product, males retiring 1941–67 from the asbestos industry and followed through 1969

Cause of death	Asbestos cement		
	Observed	Expected[a]	SMR
All causes	202	176.4	114.5
Cancer (140–205)[b]	52	30.4	171.0
Digestive (150–159)	16	11.1	141.6
Respiratory (162–163)	17	6.6	257.6
All other cancers	19	12.5	152.0
Respiratory diseases (470–527)	18	10.0	180.0
Pneumoconiosis (523–525)	10	—	—
Asbestosis (523.2)	6	—	—
Other respiratory diseases	8	10.0	80.0
All other causes	132	136.0	97.0
Cause of death	**Other asbestos**		
	Observed	Expected[a]	SMR
All causes	552	480.8	114.8
Cancer (140–205)	115	79.0	145.6
Digestive (150–159)	37	30.5	121.3
Respiratory (162–163)	41	15.1	271.5
All other cancers	37	33.4	110.8
Respiratory diseases (470–527)	49	26.8	182.8
Pneumoconiosis (523–525)	19	—	—
Asbestosis (523.2)	12	—	—
Other respiratory diseases	30	26.8	111.9
All other causes	388	375.0	103.5

[a] Based on time-age-cause specific rates for US white males.
[b] Figures in parentheses refer to rubrics of the International Classification of Diseases and Causes of Death, 7th revision, World Health Organization, Geneva, 1957.

ranging from 1.7 times expected in the lowest exposure category to 5.6 times expected in the highest (Enterline *et al.*, 1973a).

For this review, special tabulations were prepared of data from 422 men who had at one time worked with asbestos cement products. Table 2 compares the mortality experience at ages 65 and over for the 422 men who worked in asbestos cement products with that of 926 other workers in the study. For both groups the mean time-weighted dust exposure at time of retirement was about the same, 249 mppcf-years *versus* 247 mppcf-years. Also, mean age at first exposure was about the same for each group (39.8 *versus* 38.6). Table 2 shows that mortality rates among asbestos cement and other asbestos workers were very similar. This was true for all causes of death and for the selected causes shown.

Of the 422 retired workers from asbestos cement products industries, 316 had worked in the manufacture of rigid shingles and sheets while 106 had worked in the manufacture of asbestos cement pipe. Table 3 compares mortality in these two groups of workers. While mortality for all causes was about the same, death rates for cancer and respiratory disease were quite different. Men who had manufactured asbestos pipe had a very high death rate for respiratory cancer and no deaths from asbestosis; on the other hand, the excess in respiratory cancer mortality was not remarkable for men making rigid shingles, but this group contained all the deaths from asbestosis.

Although the numbers of asbestos cement workers used in Table 3 are small, one might speculate as to possible reasons for a real difference in mortality between workers in asbestos cement shingles and asbestos cement pipe. The manufacture of asbestos cement pipe was introduced into the United States only after 1928, with the result that retired men in this study started work somewhat later than did other asbestos cement workers: for pipe workers in this study the mean year of hire was 1937, as compared with 1931 for other cement workers. Thus, time since first exposure and duration of exposure were somewhat shorter for pipe workers, and this resulted in lower time-weighted exposure for this group (228 mppcf-years *versus* 256 mppcf-years), which might somehow account for the absence of asbestosis deaths. It would probably not account, however, for the high respiratory cancer mortality.

Most asbestos pipe manufactured in the United States contains crocidolite asbestos—currently 3%, but more in the past—and all asbestos cement pipe workers had exposure to crocidolite asbestos. Rigid shingles and sheets, on the other hand, rarely contained crocidolite asbestos, so hardly any of the workers making these products were exposed to it. This disparity may account for the difference in incidence of respiratory cancer between these two groups. Other factors not considered in Tables 2 and 3, such as time of retirement and type of work, do not account for the difference shown. In an earlier report it was pointed out that maintenance-service workers exposed to asbestos had very high respiratory cancer death rates and low asbestosis death rates (Enterline *et al.*, 1973b). Adjustment of the data to take account of this factor slightly increases respiratory cancer mortality rates for asbestos cement workers in comparison to other workers (Table 2) but does not change comparisons between rigid shingle and cement pipe workers (Table 3).

Table 3. Observed and expected deaths and standardised mortality ratios (SMR'S) for selected causes of death by type of product, males retiring 1941–67 from the asbestos industry and followed through 1969

Cause of death	Rigid shingles and sheets		
	Observed	Expected[a]	SMR
All causes	158	135.2	116.4
Cancer (140–205)[b]	35	23.0	152.1
Digestive (150–159)	12	8.6	139.5
Respiratory (162–163)	7	4.9	142.9
All other cancers	16	9.5	168.4
Respiratory diseases (470–527)	16	7.6	210.5
Pneumoconiosis (523–525)	9	—	—
Asbestosis (523.2)	6	—	—
Other respiratory diseases	7	7.6	92.1
All other causes	106	104.6	101.3
Cause of death	**Cement pipe**		
	Observed	Expected[a]	SMR
All causes	46	41.2	111.6
Cancer (140–205)	17	7.4	229.7
Digestive (150–159)	4	2.7	148.2
Respiratory (162–163)	10	1.7	588.2
All other cancers	3	3.0	100.0
Respiratory diseases (470–527)	2	2.4	83.3
Pneumoconiosis (523–525)	1	—	—
Asbestosis (523.2)	0	—	—
Other respiratory diseases	1	2.4	41.7
All other causes	27	31.4	86.0

[a] Based on time-age-cause specific rates for US white males.
[b] Figures in parentheses refer to rubrics of the International Classification of Diseases and Causes of Death, 7th revision, World Health Organization, Geneva, 1957.

CONCLUSIONS

Exposure to asbestos and silica dust in the asbestos cement industry produces radiographic and physiologic evidence of asbestosis and/or silicosis, and these fibrogenic effects appear to be related to the cumulative level of dust to which the worker is exposed. It is probable that a level of total dust exposure can be attained which will be unlikely to produce the pneumoconioses which have resulted from past higher levels of dust. Although to date it has been impossible to distinguish between exposures to asbestos and silica in these workers, the pulmonary function patterns suggest that the rounded nodules seen on the chest X-ray may represent an exposure predominantly to silica, and that irregular changes are associated with the effect of asbestos. It is not clear why individuals with seemingly similar exposures in this industry develop differing radiographic and physiologic patterns.

Some comparisons between asbestos cement workers and other asbestos workers are possible from studies of worker compensation records and from mortality studies. Comparisons of working populations suggest that asbestosis is less of a problem in the asbestos cement products industry than in other applications of asbestos. On the other hand, a study of a retired population shows little difference. One problem in comparing working populations is that since the asbestos cement products industry has grown rapidly, its work force is younger and has had less exposure than have other asbestos workers; this may account for their relatively lower asbestosis rate.

Regarding the effects of exposure to various types of asbestos, a mortality study of retired asbestos cement workers showed that workers exposed to crocidolite asbestos showed no asbestosis but a high respiratory cancer mortality, whereas workers exposed mainly to chrysotile asbestos showed considerable asbestosis but moderate respiratory cancer mortality. While based on small numbers, this finding supports other observations regarding the carcinogenic properties of crocidolite asbestos.

SUMMARY

The asbestos cement industry consumes the bulk of the world's output of asbestos. Workers in this industry are exposed to silica and asbestos, and they develop both asbestosis and silicosis in numbers related to their total dust exposure. It is estimated that radiographic changes of 2/1 category or above appear after a total dust exposure of 1000 mppcf-months. Asbestosis appears to be less of a problem in the asbestos cement industry than in other asbestos products industries, possibly because the former is growing more rapidly and its work force has had less dust exposure. Both chrysotile and crocidolite asbestos are used, with the latter confined almost entirely to asbestos cement pipe. Cement pipe workers appear to have a great excess in respiratory cancer but little asbestosis, whereas other cement workers have a modest excess in respiratory cancer but a considerable amount of asbestosis.

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Discussion summary

H. WEILL [1]

MINES AND MILLS (opened by Dr I. J. Selikoff)

Limited environmental data has made the estimation of past dust exposures difficult, but, in addition, the study of a working population may provide different prevalence rates of indicators of disease, such as radiographic and pulmonary function abnormalities, than if workers who have left the industry are also included. Studies in Cyprus and Canada may have had these drawbacks, whereas the Italian study, which included retired workers, was said to indicate a higher incidence of radiologic and functional abnormalities. In studying mortality, the Canadian investigation eliminated those born before 1891 as there was inadequate mortality information. It was argued that their mortality experience would be expected to differ from that for those born after 1890, as was suggested by the study of New York insulation workers. In reply, it was asserted that epidemiological studies using prevalence data are sound. In regard to the mortality study, those individuals born during 1891 are now over 80 and certainly represent workers whose onset of exposure occurred sufficiently long ago to demonstrate the full health risk. When the rates for asbestos-related diseases in the insulation workers are compared with those in the chrysotile miners and millers, the differences are so great that there can be no doubt of their validity.

Limitations in the South African study were primarily due to the small number of miners available who had twenty or more years of exposure. In view of the drawbacks of prevalence studies on working populations and the difficulty of collecting adequate environmental information, caution should be applied in the establishment of hygiene standards as a result of the reported studies of these mine and mill workers.

In the Italian study of asbestos miners there were few cases of moderate or severe asbestosis, in spite of high dust exposure. There was also a low rate of pleural calcification. Rales were most frequently heard with well-established asbestosis, but vital capacity and forced expiratory volume could be abnormal even in the absence of radiographic changes. Pulmonary diffusing capacity was found to be abnormal only when definite X-ray changes were present.

An important limitation in the study of New York insulation workers was that only those workers belonging to a trade union were included. In Devonport (UK) all dockyard workers are being studied prospectively to establish cause of death and, where possible, to relate this to the degree of asbestos exposure.

In South Africa there is an increasing annual production of asbestos, including crocidolite, amosite and chrysotile. In the Transvaal, all individuals exposed to crocidolite are also exposed to amosite, so a group with "pure" crocidolite exposure cannot be isolated. Finally, although there is an increasing number of persons exposed to asbestos, the level of exposure has been reduced.

MANUFACTURING (opened by Prof. P. C. Elmes)

The problem of grading dust exposure cannot be solved simply. Dust levels existing 20 to 30 years ago are most important and the most difficult to assess. Although manufacturers require information on maximum safe continuous levels of dust exposure the answer is not yet available because safe levels depend on the kind of asbestos, presence of other dust and smoking habits of the workers. At

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present, the effects of exposure to asbestos cannot be detected early enough, so the epidemiologist must determine dust-disease relations for different jobs in spite of the limitations of the environmental data.

It was emphasised that, particularly for monitoring purposes, workers handling asbestos should be examined periodically, not just while they are working but perhaps for the rest of their lives. The examination could be simple, including a brief history and physical examination, chest X-ray and forced expiratory volume and vital capacity. The frequency of these examinations might be once every two years. When asbestosis is recognised, immediate marked reduction of dust levels must be made.

In the asbestos-cement industry in the New Orleans area mixed radiographic patterns including both small rounded and irregular opacities occur. It was suggested that the prognosis was more unfavourable in those cases with irregular opacities in the lower lung, and it is in these cases that pleural changes are likely to be marked. Pulmonary function was also worst in the group with irregular opacities and was further reduced by the presence of pleural changes. Limited data from a German study indicated that cement dust did not have an accelerating effect on the development of asbestosis; however, free silica was probably not used in these processes, whereas it was in the American plants studied. In a study in South Africa, where 869 out of 1490 employees in the asbestos-cement industry have had more than three years' exposure, twenty-five were found to have asbestosis and eleven showed calcified plaques. It was also reported that baboons exposed to asbestos-cement dust showed diffuse interstitial fibrosis but no asbestos bodies, perhaps because of the low fibre exposure. In a Belgian asbestos-cement factory less than one per cent of workers have abnormalities, but most of these workers had exposure for less than twenty years. As exposure increased beyond this time, radiographic abnormalities increased as well.

A film of a new method for the manufacture of asbestos textile using chrysotile fibre was shown. The spinning is performed wet. Since the major dust exposure in the textile industry is in carding and spinning, this new procedure will eliminate the major dust hazard in this part of the industry.

In further general discussion, it was suggested that available evidence indicates the need to restrict the use of crocidolite fibre in manufacturing. An example given was that the use of loose crocidolite will be forbidden in Holland in 1973, and when it is used by imbedding, as with asbestos-cement products, it will be allowed only in concentrations of up to 10%. There was disagreement as to whether asbestos-cement products could be made without crocidolite, without loss of the advantages given by this fibre.

Past dust exposure indices have been validated by the dust dose-disease response relationships which have been demonstrated, including for respiratory cancer. It was concluded that time-weighted measurements based on midget impinger counts of asbestos dust are the best available for relating asbestos to disease. Alternatives to this method of evaluating past environmental conditions and producing dose-response relationships have yet to be validated in this way.

CANCER IN RELATION TO TYPE OF FIBRE, DOSE, OCCUPATION AND DURATION OF EXPOSURE

Chairman — W. Gardner

Rapporteur — C. E. Rossiter

Cancer in chrysotile mines and mills

J. C. McDONALD [1]

In my review of asbestosis in chrysotile mines and mills, the history and present state of the industry were outlined (McDonald [2]). Special attention was given to epidemiological inquiries which attempted to define exposure-response relationships, and reference was made to measurements or estimates of dust and fibre concentrations which have prevailed in the working environment. Cancer was recognised only fairly recently as an occupational hazard for asbestos workers, and the conceptual and practical problems involved in its investigation are more complex than for pulmonary fibrosis. The fibrogenic mechanism in asbestosis, though obscure, can still be investigated in a relatively direct manner; whereas carcinogenic activity can be examined only on the basis of questionable assumptions as to latent period, the role of co-carcinogens and the interaction of time and dose. Statistical difficulties arise in attempting to separate duration of exposure from age at and year of death. Since asbestosis does not occur, for all practical purposes, in persons not occupationally exposed, controls are scarcely necessary to determine either causal or dose relationships for this disease. In contrast, respiratory and gastro-intestinal cancers are common in the general population, and several etiological factors, other than asbestos, are already known or suspected. Moreover, the diagnosis of these tumours is heavily dependent on autopsy, and whether or not an autopsy is performed is in turn related to social influences often correlated with occupation. For these reasons the objectives of this review are more limited than for asbestosis. The main question is whether or not there is any cancer excess in chrysotile miners and millers; incidence can then be considered in relation to amount of exposure and evidence of co-factors.

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[2] See p. 155 of this publication.

LUNG CANCER

The first systematic but limited study of lung cancer in chrysotile miners and millers was published by Braun & Truan (1958). In this inquiry, made in eastern Quebec, nine definite and three probable cases were observed in a defined cohort, compared with six expected from Provincial rates. Eight years later, Kogan *et al.* (1966) reported that mortality from lung cancer among mine and mill workers in one of the Russian mining cities, during a 10-year period, was considerably higher than in the rest of the population. The increase was by a factor of 1.9 for miners, 3.1 for millers and 2.3 for factory workers. The methods of data collection and analysis were not described in any detail.

Largely as a result of the recommendation made after the 1964 New York conference (NYAS, 1965) that priority should be given to study of the effects of pure exposure to various fibre types, a full-scale investigation in the Quebec industry was begun in 1966 and is still in progress. A major aspect of the inquiry is concerned with mortality in a cohort of some 12,000 persons, comprising all those born between 1891 and 1920 who had ever been employed in any part of the industry for more than one month. Various indices of exposure were calculated for each employee, using the work history and reasonably good environmental measurements (Gibbs & Lachance, 1972). An index of total dust exposure was obtained by summing the products of dust level, in millions of particles per cubic foot (mppcf), and number of years at that level.

Two analyses of mortality in this cohort have been made so far, the first based on 2457 deaths up to November 1966 (McDonald *et al.*, 1971), and the second on a further 813 deaths up to the end of 1969 (McDonald *et al.*, 1973). The results of both analyses were essentially the same, so the combined

findings for deaths by malignant disease are presented in Table 1. Age-corrected death rates for all malignant neoplasms were somewhat raised in the two highest dust exposure groups, the increase being mainly due to tumours of the respiratory tract and, to a lesser extent, of the gastro-intestinal tract. There were, in all, 135 respiratory cancers in the series, 134 in men. Included in this total were five pleural mesotheliomas. The number expected in men was 139 on the basis of Quebec death rates, or about 93 on the basis of estimated rates for the mining region. This suggests that the mortality from respiratory cancer in chrysotile workers was, at most, 45% above expectation. Table 1 indicates that any excess was virtually confined to persons with a dust index above 200 mppcf, and most evident in those with exposure above 400 mppcf. The number of subjects becomes very small, but men in this highest category of exposure, and employed for 30 years or more, experienced about five times the mortality rate from respiratory cancer of those in the lowest exposure group, employed for less than one year.

About half the persons in the cohort of 12,000 had been X-rayed while at work, but as these tended to be the younger men, the number of deaths was only 785. An analysis of deaths by cause in those with and without radiographic evidence of asbestosis indicated that most of the excess mortality was in men with parenchymal changes and attributable to respiratory disease, including cancer.

In the Quebec study, equivalent average death rate was used as a parameter for age correction; this proved unsatisfactory, in that it did not allow for interaction between length of exposure and length of survival. Review of the data did not suggest the likelihood of serious error, but a provisional reanalysis has been made by another method not subject to this problem, by which the number of expected deaths in each exposure category could be estimated. The total number of expected male deaths from respiratory cancer in the cohort, estimated by this method, was 95 compared with 136 observed. The excess became evident somewhere between 100 and 400 mppcf-years and was clearly present above this level.

Table 1. Equivalent average death rates for malignant disease, to end of 1969, per 1000 men born 1891–1920 and employed in the chrysotile mines and mills of Quebec

	Dust index (mppcf-years)						
Number of men	<10 2810	10–100 3329	100–200 1124	200–400 1007	400–800 837	800 + 585	All 9692
All malignant neoplasms	57.9	60.8	53.8	40.6	67.1	79.3	58.0
Gastro-intestinal and respiratory neoplasms	28.3	26.7	32.1	27.1	47.7	60.8	31.2
Other malignant neoplasms	29.6	34.1	21.7	13.5	19.4	18.5	26.8
Bronchus, trachea and lung[a]	10.3	13.1	13.4	15.5	21.4	32.1	14.2
Oesophagus and stomach	13.5	7.0	10.0	6.6	18.8	20.2	11.0
Intestine and rectum	4.5	6.6	8.7	5.0	7.5	8.5	6.0
All gastro-intestinal	18.0	13.6	18.7	11.6	26.3	28.7	17.0
All causes	365.0	354.9	353.9	313.2	323.1	394.6	353.7

[a] Including malignant mesothelioma (5 pleural, 0 peritoneal).

Table 2. Comparison of findings from cohort studies of mortality in chrysotile mines and mills in Northern Italy and Quebec

	N. Italy	Quebec
Number of men	1,098	11,107
Number and proportion traced	998 (91%)	9,692 (87%)
Number and proportion dead	270 (27%)	2,950 (30%)
Number and percentage distribution of deaths by cause		
Tuberculosis	21 (8%)	250 (8%)
Respiratory disease (excl. TB and malignancies)	34 (13%)	369 (13%)
Cardiovascular disease	71 (26%)	1,058 (36%)
Accidents	59 (22%)	369 (13%)
Malignant disease	30 (11%)	519 (18%)
mesothelioma	0 (–)	5 (0.2%)
other respiratory	6 (2%)	129 (4%)
gastro-intestinal	11 (4%)	152 (5%)
other	13 (5%)	233 (8%)
Other causes	44 (16%)	169 (6%)
Not known	11 (4%)	216 (7%)
Total	270 (100%)	2,950 (100%)

A directly comparable cohort study was undertaken by Vigliani [1] and his colleagues on the chrysotile mine and mill workers of Ballangero in Northern Italy. The number in the cohort was almost exactly one-tenth that of the Quebec study, and the results, summarised in Table 2, are quite similar. The full analysis and the conclusions are not yet available, but there appears to be even less evidence of respiratory cancer in the Italian workers than among those in Quebec.

In their review of asbestosis in Rhodesia, Gelfand & Morton (1970) examined the scanty data available on lung cancer and were unable to detect any relationship between the two diseases. They expressed doubt, however, about the reliability of this conclusion. No information relating to the incidence of lung cancer in chrysotile workers has been reported from South Africa, Cyprus or any of the other mining areas.

Interaction between asbestos exposure and cigarette smoking is an attractive hypothesis for which there is some evidence in insulation workers (Selikoff *et al.*, 1968). A prospective investigation of this point is currently under way in Quebec. A retrospective inquiry in which cases of lung cancer in chrysotile workers were matched with cases from the general population showed an excess of non-smokers and light smokers in the asbestos group (Manfreda & Eyssen [2]). These results certainly do not support the concept of synergism, but they do not wholly exclude it.

MALIGNANT MESOTHELIAL TUMOURS

The number of cases of malignant mesothelioma directly associated with chrysotile mining and milling is very small. Some effort has been made to identify cases in six countries, together responsible for 92% of the world's chrysotile production, and eleven cases in all (nine probable and two doubtful) have come to light. It may be seen in Table 3 that the six countries have been in production for substantial periods of time, during which many thousands of workers have been employed. Almost all known cases have been in Canada where ascertainment has been intensive both in the general population and among past employees of the mining industry (McDonald *et al.*, 1970). An equally careful study of Italian miners and millers failed to discover any cases; although one doubtful neighbourhood case was found. Because of local circumstances in Cyprus, South Africa and Rhodesia, cases may have been missed, and only one neighbourhood case (in Cyprus) was discovered. Nothing is known for certain about the situation in the USSR, the largest producer of chrysotile, or in the USA, also a major producer. Kogan [3] has stated that he knows of no cases in the Russian industry; and no published or

Table 3. Recorded deaths from malignant mesothelioma associated directly or indirectly with chrysotile production

Country	Year commercial production began	Estimated output for 1970 (short tons)	Approximate number in current work force	Recorded deaths for mesothelioma		
				Occupational	Household	Neighbourhood
USSR	1885	2,200,000	not known	0	0	0
Canada	1878	1,659,084	8000	6	3[a]	0
Rhodesia	1908	150,000	8000	0	0	0
Italy	c. 1919	120,000	250	0	0	1
South Africa	c. 1910	50,000	1350	0	0	0
Cyprus	1904	28,000	450	0	0	1
				6	3[a]	2

[a] One of these cases had also been employed by the industry in geophysical exploration, but his home exposure was considered more significant (see Table 4, case 6).

Note. China, USA, Swaziland, India, Japan and Yugoslavia have a combined production of about 380,000 tons, but no information has been found for these countries.

[1] Personal communication.

[2] Unpublished data.

[3] Personal communication.

unpublished reference has been found to any cases related to the American industry.

Information concerning the eleven cases is set out in Table 4. The seven men employed in the Quebec industry comprise four cases considered definite (cases 1, 2, 3 & 5), one (case 6) whose domestic exposure was probably more relevant than his occupation, and two in which there was an element of doubt: case 4 was not accepted by the Canadian Mesothelioma Panel of Pathologists, and case 7, although acceptable pathologically, had only an uncertain history of having worked for one of the Quebec mining companies during the 1890's. This exposure, if responsible for his death at 91, would imply a latent period of about 77 years! Cases 1 to 5 were in the cohort study of mortality, and so we have information on the duration and intensity of their dust exposure. The men tended to have been long-term employees, but there was little or no relation to dust index. Three cases, one man and two women, were from families in which the father worked in the mills, so it may be presumed that they had been subject to indirect exposure since infancy. In general, these patients died at an earlier age than those occupationally exposed.

Excluding case 6, four of the six cases occupationally exposed had worked in Thetford Mines and two in Asbestos, these numbers being exactly proportional to the number of past employees in these two areas. However, dust exposure has been consistently higher at Thetford Mines, and radiographic changes, especially pleural thickening and calcification, have been much more prevalent there (Rossiter *et al.*, 1972). Pleural calcification is common in older employees at Thetford and is virtually unknown at Asbestos. Whatever is responsible for pleural thickening and calcification does not, therefore, seem to be related to mesothelioma. In the Canadian mesothelioma survey (McDonald *et al.*, 1970) it was shown that the smoking habits of cases and controls representative of the general population were almost identical, and that both groups had smoked much less than had most of the cases of primary lung cancer. It is therefore most unlikely that cigarette smoking is a causal factor in mesothelioma.

Table 4. Age, sex and dust exposure for all recorded cases of malignant mesothelioma directly or indirectly associated with chrysotile production

Case	Sex	Age at death	Year of death	Years employed by industry	Dust index (mppcf-yrs)	First exposure to death (yrs)	Exposure
1	M	41	1956	15.7	73	21	mine and crusher
2	M	60	1964	44.7	358	45	mill
3	M	64	1956	3.2	102	45	mill
4[a]	M	57	1967	30.4	485	31	mill
5	M	73	1967	19.3	53	30	mine
6	M	32	1968	10.0	NK[b]	32	geophysical exploration; father also worked for the industry
7	M	91	1970	"few"	NK	c. 77	mine and mill
8	F	42	1967	0	NK	42	father worked for the industry
9	F	51	1970	0	NK	51	father worked for the industry
10	F	NK	NK	0	NK	NK	lived 7 years near Cyprus mine
11[a]	NK	NK	NK	0	NK	NK	lived in village 3 km from Italian mine

[a] Doubtful case.
[b] NK—not known.

Evidence to incriminate chrysotile exposure, as it occurs in mining and milling, in the etiology of mesothelial tumours clearly depends on the Quebec data. Having regard for the fact that well over 30,000 persons have been employed in the Quebec industry, and that many times this number have been exposed indirectly at home or in the general environment, the number of known cases is very small. Moreover, the probability of diagnosis for industry-related cases must surely be higher than for the general population. Even so, the eight definite cases are at least four times expectation, and it seems unlikely that this excess can be explained completely by better ascertainment.

GASTRO-INTESTINAL CANCERS

In the Quebec cohort, the standardised death rate from gastro-intestinal malignancies, 170 per thousand, accounted for nearly one-third of the rate for all malignant neoplasms (Table 1). Overall, there is a suggestion of an increased rate for men with a dust index above 400 mppcf. However, the difference is less than 2:1, and the site distribution has not followed any consistent pattern. In an analysis of deaths up to 1 November 1966 (McDonald *et al.*, 1971), an increase of similar order was noted, mainly due to cancers of the intestine and rectum. Addition of deaths to the end of 1969 maintained the gradient, but it was then more evident in cancers of the oesophagus and stomach.

It has been seen (Table 2) that Professor Vigliani's results from Ballangero parallel those from Quebec and do not give further evidence of an excess of gastro-intestinal cancer. No information on this disease is available from Cyprus, Rhodesia or South Africa, and the Russian data have not been analysed from this point of view.

SUMMARY

Evidence for a link between malignant disease and occupational exposure in the chrysotile producing industry is not strong, despite the fact that men dying now were exposed for many years to much higher dust concentrations than prevail today. More results would of course be useful, and no doubt research methods can be improved; nevertheless, the investigation of chrysotile miners and millers has been on a larger scale than that of other asbestos workers and generally with much more information on levels of exposure. The results appear to show that there is a modest increase in respiratory and probably gastro-intestinal cancer in this industry, most serious in those who have been heavily exposed over long periods. This hazard would probably not be detectable at exposure levels below about 200 mppcf-years, i.e., 50 years at 4 mppcf. The fibre equivalent for this figure has not been established, and Quebec data indicate that it may be difficult to arrive at any single conversion factor.

The link with malignant mesothelioma is also weak and there is no evidence of an association with peritoneal tumours. Chrysotile-related tumours are too few to permit any firm conclusion on exposure-response. The fact that in Canada there have been cases of mesothelioma found after occupational and domestic but not purely neighbourhood exposure suggests that the low dust levels prevailing in mining communities have not been sufficient to cause the disease.

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Malignancy in relation to crocidolite and amosite

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The asbestos areas of South Africa are of particular interest as it is only there that relatively large numbers of people have been exposed to a single type of asbestos. The map (Fig. 1) indicates the relationships of these areas to one another. It is, however, important to study these areas in more detail than has been done in the past.

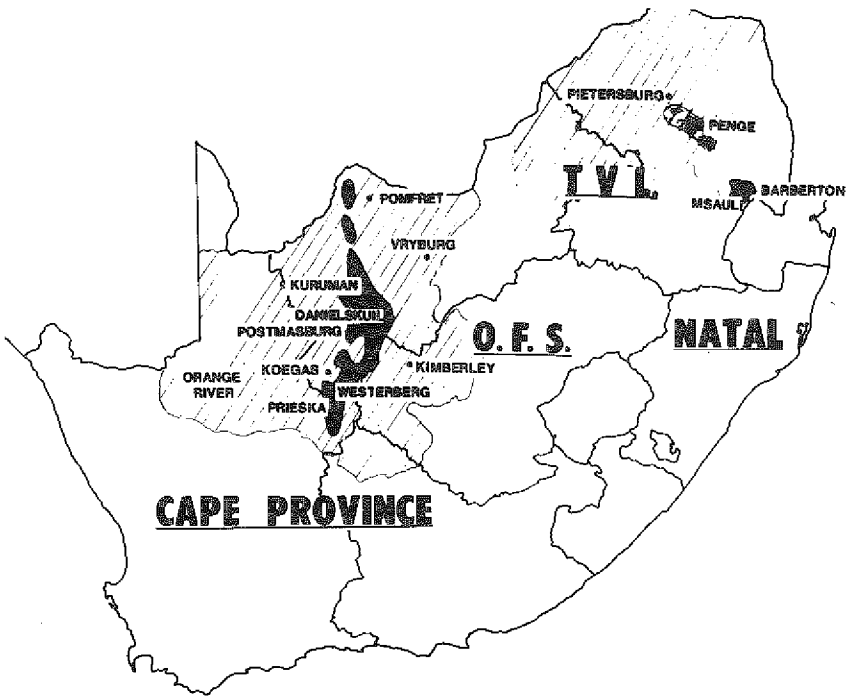

Fig. 1. Distribution of asbestos areas.

Cape crocidolite is mined in an area which extends from the region of Prieska on the Orange River to Pomfret near the Botswana border. Although asbestos was mined and milled at Prieska, the asbestos in the south now comes from Koegas and Westerberg. In the north, mining operations are carried out at Danielskuil and, sporadically, at Postmasburg. In the middle of this asbestos area is the town of Kuruman. Other minerals such as manganese and iron are also produced. The manganese mines and a larger iron mine are on the windward side of the asbestos areas and of the towns associated with these areas; but iron deposits are present on both

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sides of the asbestos areas, and the host rock of Cape Blue asbestos is banded ironstone or jasper.

The dust most commonly found in the atmosphere of the area is laterite (Sleggs, 1969 [1]), which is non-fibrous but which contains the same minerals as are found in crocidolite asbestos. Crocidolite, amosite and chrysotile are mined and processed in the Transvaal. Although it is possible to find Transvaal crocidolite without amosite near Pietersburg, this asbestos field usually has a seam of amosite which is found above the crocidolite seam. In certain areas the seams are in close proximity, being found as duplex seams. Although the amosite is of poor quality and is not mined, crocidolite mining operations can create a dust cloud containing both crocidolite and amosite fibres.

The Transvaal crocidolite field runs south-east from Pietersburg to Penge, where a group of large mines produces amosite asbestos. Other minerals are also found; but a manganese deposit is recorded only on the west side of Pietersburg. There are scattered chrysotile mines to the south-east of the amosite area, the largest being at Msauli near the Swaziland border.

Analyses obtained by Kuyper & Hodgson [2] indicate the main differences in the trace elements found in the asbestos from the different areas. The manganese content increases from the south of the Cape crocidolite field to the north and through the Transvaal crocidolite field to the amosite area, where it is approximately 5%; the sodium content decreases in the same way. As far as can be determined these analyses were carried out on the cobs of asbestos, and not after milling.

The processing of asbestos has changed over the years, the pertinent factor being the types of beater and liners used. Up to 15 years ago the Kuruman Blue Mines used beaters made of a manganese steel alloy, and this type of steel was used in the Cape Blue Asbestos Company's mines until about 6 years ago (Baunach & Dougherty, 1972 [3]). These beaters had to be changed frequently because of excessive wear; and the present beaters, most of which are made of a nickel-chrome-molybdenum-steel alloy, need to be replaced only after a much longer time. On some mines, high carbon-chrome alloys are being used. Thus the trace mineral content of the asbestos produced during the last few years will be different from that produced some 6 to 10 years ago.

The tonnage of asbestos produced has increased. In 1940 some 18,000 tons of amosite were produced, compared with 7000 tons of Cape crocidolite and 3000 tons of Transvaal crocidolite. In 1950 the approximate figures were 42,000 tons of amosite, 15,000 tons of Cape crocidolite and 15,000 tons of Transvaal crocidolite. In 1960 the amounts of amosite and Cape crocidolite produced were approximately the same, namely 67,000 tons, but the tonnage of Transvaal crocidolite produced had dropped to 11,000. This trend continued until in 1970 approximately 98,000 tons of amosite were produced as compared to 131,000 tons of Cape crocidolite and 5000 tons of Transvaal Blue asbestos.

It is reasonable to assume that the labour forces on these different mines were related to the tonnage produced, and that therefore for many years more people were exposed to amosite asbestos than to Cape crocidolite, and that some 20 years ago there were as many people mining and processing Transvaal Blue as there were working with Cape crocidolite.

Although in earlier days Cape crocidolite was brought to central milling points by tributors, the production of this type of crocidolite today is by relatively large mining operations such as are found at Koegas, Danielskuil, Kuruman and Pomfret. Under the tribute system there was no legislation to control such small groups of workers; however, there were undoubtedly many in the northern Cape who took part in such tribute operations, since there are many outcrops of asbestos on the farms in this area. In the Transvaal, the tribute system is still to be found in the crocidolite area; it is thus impossible to define the population presently at risk in the Transvaal, and that at risk some 20 years ago in the Cape.

In 1971 the labour force on the asbestos mines totalled 20,600 people (Miners' Medical Bureau [4]) of which 10,440 employees were to be found in the Transvaal and 10,161 in the Cape Province. At the Penge amosite mine there is a working complement of 8500 employees, at Koegas 1250, and at Pomfret 1250. In the Cape some of the employees are of mixed race, whereas in the Transvaal the employees are either Caucasian or Bantu, the latter making up the greater part of the labour complement. In

[1] Personal communication.
[2] Unpublished data.
[3] Personal communication.
[4] Personal communication.

general, it may be said that the employees of mixed race smoke more than do the Bantu; however, some of the latter do smoke cigarettes or pipe tobacco, and many use snuff.

The first set of figures that we have studied consists of the records of the Asbestos Tumour Reference Panel and the Registry of such tumours in the National Research Institute for Occupational Diseases. A total of 375 cases of mesothelioma has been confirmed and registered by the Panel. Of these, 360 were pleural mesotheliomas. The exposure to asbestos of these patients is indicated in Table 1.

Table 1. Mesothelioma of pleura and asbestos exposure—360 cases

		No. of cases	Total nos. of cases	Per cent of total
Mining	Cape Blue	45		
	Cape Blue + manganese	23		
	Cobbing	15		
	Amosite	4		
	Transvaal Blue	1	88	24.4
Industrial	Railways (SA)	13		
	Other	21	34	9.4
Environmental	Definite	36		
	Possible	40		
	Occasional	7	83	23.1
No known exposure			47	13.1
History unknown			108	30.0
			360	

Eighty-eight of the cases had been associated with the mining industry, and of these the majority had worked in crocidolite mines of the northern Cape. Only four cases had had service on the amosite mines, but one of these worked in the northern Cape, and the industrial history of the other is far from complete; thus there are two who have definitely had amosite service only. One patient had only worked on a mine in the Transvaal crocidolite field for approximately 3 weeks, but he lived some 800 yards from the mill.

Environmental exposure is confined to the northern Cape. It is, however, noticeable that the number of cases of pleural mesothelioma without any history of asbestos exposure is increasing and now stands at 47. Unfortunately, for a large number of these patients there is no occupational or environmental history.

Apart from the data in Table 1, the following points have emerged from analysis of the cases of pleural mesothelioma:

(1) Mesothelioma of the pleura occurs predominantly in the south and central parts of the Cape Blue asbestos fields.

(2) Only three persons with mesotheliomas had had service on the most northern mine, although the working complements of Koegas in the south and of Pomfret in the north are approximately the same, namely about 1250.

(3) Two cases of mesothelioma have been found in mine workers with amosite exposure only. Two further cases had had service at Penge, one with subsequent exposure to Cape Blue asbestos; no further details of exposure are available for the other.

(4) One patient was exposed only in the Transvaal crocidolite fields.

(5) The mesotheliomas appear to occur in family groups, but it is not possible to exclude the factor of exposure to the same environment.

The second set of figures used comes from the analysis of autopsy examinations of the cardio-respiratory organs removed in accordance with the Pneumoconiosis Act. The asbestos mining industry was listed in 1956 as a controlled dusty occupation; thus the medical officer is obliged to remove the cardio-respiratory organs from all patients who have been in his care and died and whom he suspects of having pneumoconiosis or whom he knows to have worked in a controlled occupation, and to send these organs to the Miners' Medical Bureau. The nearest relative must give consent for the autopsy examination, if he or she is readily available.

However, when a miner leaves the mines it is possible that he may be in receipt of full compensation and that there is thus no advantage to the family if the nearest relative gives permission for an autopsy Many undoubtedly retire from the industry to isolated farming areas in the different homelands, and they would not usually seek help from one of the many mission hospitals in the area unless definite symptoms were present. Many still prefer to consult their own herbalists or witch-doctors rather than to seek help at a hospital or from the State Health service. A State Tuberculosis Service operates both in the Cape and in the Transvaal, and the personal interest in the problem of asbestosis shown by Dr Sleggs in the northern Cape has been of great assistance.

Since 1959, 1165 specimens have been examined, and of these 94 showed the presence of malignant disease in the cardio-respiratory organs. Sixty-one of these were bronchogenic carcinomas and 33 were mesotheliomas.

The exposure records have been more reliable since 1965, and 710 specimens had been examined up to the end of 1971. Table 2 shows the distribution of the cases of bronchogenic carcinoma according to the type of asbestos exposure, in comparison with cases found among gold miners.

Table 2. Bronchogenic carcinoma in cardio-respiratory organs of asbestos and gold miners. 1965–71

	Number examined	Bronchogenic carcinoma	Percentage
Asbestos			
Cape Blue	387	11	2.8
Amosite	262	9	3.4
Transvaal Blue	61	6	9.8
Chrysotile	74	3	4.1
Gold			
Bantu	3435	67	2.0
Bantu 40+ yrs.	1611	56	3.5
Caucasian	2388	182	7.6
Caucasian 40+ yrs.	2257	181	8.0
All miners	3868	237	6.1

The significance of the figures quoted in this table should be assessed with the following considerations in mind:

(1) Not all deceased miners are represented.

(2) The series has been weighted by ex-employees who have attended mission hospitals or other health services.

(3) Autopsies are more likely to be carried out in a mission hospital if the medical officer suspects tuberculosis. Tuberculosis is known in the mining community to be a compensatable disease in certain circumstances.

(4) In sixteen of the twenty-nine cases diagnosed in 1965–71 there is a history of exposure to other minerals.

(5) In fourteen of the patients a smoking history is available. Only two were non-smokers.

If the figures are significant (and this has not yet been proven) then it is suggested that the following conclusions may be drawn from them:

(1) The percentage of cases of bronchogenic carcinoma is similar in those exposed to Cape crocidolite and to amosite.

(2) Although the series are small the percentages of cases of bronchogenic carcinoma found among those exposed to Transvaal crocidolite and to South African chrysotile are high.

(3) Results from a comparable group of gold miners whose cardio-respiratory organs were examined show that the percentages of cases of bronchogenic carcinoma are similar to those found in the asbestos areas.

It appears therefore, that cases of bronchogenic carcinoma occur in all South African asbestos areas. Mesothelioma is confined mainly to the south and central parts of the Cape Blue asbestos fields. In relating these findings to the differences in asbestos composition it may be important to note that the manganese content of the asbestos increases from the southern part of the Cape asbestos fields to the amosite area. Manganese does increase the breakdown of benzpyrene in low concentrations, but in higher concentrations it inhibits it.

Much has been done to improve conditions in the mines and mills of the asbestos industry in South Africa and to prevent asbestos from polluting the atmosphere of the towns. It is hoped that with the introduction of cumulative dust exposure record cards a prospective study on the relationship between asbestos and malignant disease will be made possible.

SUMMARY

The distribution of tumours in the different asbestos areas of South Africa is presented, as a possible clarification of the association ascribed to asbestos and malignancy. Two series of cases are given: the first, 360 pleural mesotheliomas, and the second, an analysis of bronchogenic carcinoma found in 784 cardio-respiratory organ samples submitted from the asbestos fields. These figures have been compared with a similar series in gold miners.

Mortality and morbidity of employees of anthophyllite asbestos mines in Finland

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Anthophyllite asbestos, which belongs to the amphibole group on the basis of its crystal structure, has a number of characteristics which differentiate it from the other asbestiform minerals. It has a strong resistance to acids (Speil & Leineweber, 1973) which may explain its resistance to decomposition in the lungs. Anthophyllite asbestos is relatively rigid, despite its small average cross sectional area of fibres. It cannot be woven like the common soft chrysotile asbestos; however, its rigidity is a useful property in, for example, the wet-processing techniques employed in the manufacture of asbestos-cement products or in making fine porous filters for rapid filtration flow. This rigidity may be related to the early appearance of lung fibrosis in animal experiments with anthophyllite asbestos (Holt *et al.*, 1965).

Anthophyllite asbestos is used mainly in conjunction with other asbestos types in the manufacture of asbestos cement products, and as an insulation material, since it is a poor conductor of electricity and is strongly resistent to heat and chemicals.

Compared to the world's total production of approximately 4.5 million short tons of asbestos, the anthophyllite production is small, i.e., about 10–20,000 short tons annually. Most of it comes from the Finnish asbestos mine at Paakkila, although some anthophyllite has also been mined in Georgia and the Carolinas in the USA, and in Kenya (Hendry, 1965).

Despite the fact that Finland is a country with a sparse population and a relatively low degree of industrialisation, the occurrence of asbestos in the lungs of Finns seems to be unusually frequent. In an earlier study, asbestos bodies were found to occur in about 60% of adult autopsy cases in this country (Meurman, 1966). This result has recently been confirmed, and now asbestos fibres have been detected in almost 100% of subsequent adult autopsy cases (Huttunen, 1972[4]). Pleural plaques are also found unusually commonly both at autopsy and as a roentgenologic finding—especially in the population living in the neighbourhood of the asbestos mines (Kiviluoto, 1960; Raunio, 1966). There is also an unusually high incidence of lung cancer in Finland (UICC, 1970).

On the other hand, the smoking of cigarettes is and has long been a very common habit (Pedersen *et al.*, 1969).

The present paper gives a short summary of the main results of a study on the mortality and morbidity of the working population of the Finnish anthophyllite asbestos mines. There are two mines in commercial use in the asbestos area in the province of North Savo: the Paakkila mine has been in industrial use since 1918; but the other mine, Maljasalmi, some 10 kilometres from Paakkila, has been utilised only sporadically since 1940.

The personnel registry of the working population of these mines has been fairly well kept since 1936. Our analysis covered the period between 1 January 1936 and 1 July 1967. All persons who had worked for three months or more were included. Controls, matched for age and sex, were obtained from the electoral lists of another municipality some 60 kilometres to the west of the mines, in an agricultural area containing no mines or industrial plants.

The mortality statistics were based on death

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[4] Unpublished data.

certificates, and the data for morbidity on mailed questionnaires. The risks for different respiratory symptoms and diseases were evaluated in terms of prevalence rates and relative risks. The rates were adjusted according to smoking habits, using the control group as the reference population.

RESULTS

The number of complete follow-ups of asbestos company employees known to have worked for at least three months during the 30-year period from 1936 to 1967 was 1041. Some 4.7% of the cases could not be traced (Table 1).

Table 1. The number of asbestos mining company workers known to have worked for three months or longer between 1 January 1936 and 1 July 1967, by follow-up status

	No. of workers	
Complete follow-up	1041	
died before 1 July 1967		248
died between 1 July 1967 and 20 May 1969		6
alive, questionnaire received		787
Lost for follow-up	51	
Total	1092	

The results of the mortality study are shown in Table 2. Of 248 deceased asbestos employees, 21 showed lung cancer as their principal cause of death; in the control group the corresponding number was 13, which closely coincided with the expected number (12.6) based on the national mortality statistics.

Table 2. The number of deaths by cause among asbestos employees and controls

Cause of death	Cases		Controls	
	Male	Female	Male	Female
Cancer of lung	21	—	13	—
Pulmonary TB	32	4	15	2
Cancer of digestive organs	7	—	8	1
Other cancers	9	1	7	2
Cardiovascular deaths	71	12	91	9
Cerebrovascular deaths	16	5	15	9
Trauma, veneficium or suicide	30	—	41	—
Asbestosis	10	3	—	—
Other (remaining) deaths	20	7	26	9
All causes	216	32	216	32

Although the excess of lung cancer deaths, 21 *versus* 13, is not significant, a proportional mortality study of the asbestos deaths against the national population of Finland showed a significant excess (observed 8, expected 2.4) amongst those workers with more than 10 years' exposure to anthophyllite.

The smoking habits of the deceased cases and of their controls were not obtainable; however, the smoking habits of the living asbestos employees were established, and it was found that 66.7% of these were smokers, *versus* 60.1% of the controls. Assuming that similar differences persisted among the dead cases and controls, it is calculated that the relative risks of lung cancer are as follows:

(a) for a non-smoking asbestos worker, 1.4;
(b) for a smoker without asbestos exposure, 12; and
(c) for an asbestos worker who smokes, 17;

in terms of unit risk to a non-smoker without asbestos exposure, assuming multiplicability between the risk due to asbestos and risk due to smoking.

Asbestosis was the primary cause of death in 13 cases among the asbestos employees. Not a single case of mesothelioma could be established with certainty, although one was suspected on the basis of his chest X-ray and long exposure to asbestos dust.

Of the 32 cases who, according to the death certificates, had died of pulmonary tuberculosis, there were 10 in which, in our opinion, evidence was incomplete—it is possible that some of these died of lung cancer or other cancers, even of mesothelioma. Information as to the cause of death was based on autopsy in 35 cases of asbestos employees and in 25 cases of controls.

The number of deaths on which the results are based is rather small; this is especially true of those cases who had worked for 10 years or more, of which there were only 43. Of these only 34 had been miners or mill workers with heavy exposure. Of the nine cases who had been employed for 20 years or more, six had asbestosis at the time of death, and five of the nine died of lung cancer.

There was no excess risk of cancer of the digestive organs or of other cancers, as can be seen from the statistics in Table 2; this was confirmed by national mortality figures.

Our opinion, based on the above statistics, is that it is still too early to draw any far-reaching conclusions concerning the association of malignancy and anthophyllite asbestos exposure in Finland. The same material should be re-examined in 5 to 10 years' time, when further follow-ups will be possible; several cases have died of lung cancer since the study was terminated. However, no cases of mesothelioma have appeared so far.

The living asbestos employees were analysed on the basis of three variables: state of health, presence or absence of cough and dyspnoea. The main group of living asbestos employees contained many workers who cannot be considered actual asbestos workers, since their working conditions were not particularly dusty, e.g., office or forest workers. Thus, a high-risk group of actual miners and mill workers with an exposure time of 10 years or more was selected. The main group consisted of 787 cases, and the high-risk sub-group of 110 cases. The different smoking habits among the asbestos employees and their controls necessitated an adjustment of the prevalence rates of respiratory symptoms according to the daily consumption of cigarettes.

State of health was evaluated by the subjects themselves. These indicated no effect of asbestos exposure in either the main group of asbestos employees or in the sub-group with heavy exposure. We can conclude that an estimation of the general state of health by the subjects themselves is too subjective a method to give any reliable results.

The occurrence of a cough shows a direct correlation to the amount of exposure to asbestos (Table 3). Cough was twice as common (relative risk 2.2) in the whole group of asbestos employees as among their controls. In the group with heavy exposure the occurrence of cough was more than four times as common (relative risk 4.4) as among their controls.

To evaluate the occurrence of dyspnoea among the asbestos employees and their controls, only cases who showed this condition at rest were accepted for analysis. As is shown in Table 4, dyspnoea was more common among asbestos workers than among controls. There was no trend in the prevalence of this condition with increasing exposure to asbestos. The prevalence of dyspnoea is higher for asbestos workers than for controls in every smoking category, even if this risk does not increase with an increasing number of cigarettes smoked. The excess of dyspnoea can be assumed to be due to asbestos and might indicate more or less advanced asbestosis.

Table 3. Prevalence rate (per cent) of cough by exposure to asbestos and smoking habits

No. of cigarettes smoked per day	Total cases		Heavy exposure	
	Employees	Controls	Employees	Controls
None	17	7	59	10
≤15	32	13	57	14
>15	36	20	57	19
Total[a]	27	12	58	13

[a] Adjusted for the smoking distribution of controls as standard.

Table 4. Prevalence rates (per cent) and relative risks of dyspnoea by smoking habit for asbestos employees and controls in terms of unit risk for controls

No. of cigarettes smoked per day	Employees	Controls	Relative risks
None	20	4	5.6
≤15	23	8	3.0
>15	13	8	1.8
Total[a]	19.9	6.1	3.3

[a] Adjusted for the smoking distribution of controls as standard.

SUMMARY

A study of the effects of anthophyllite asbestos on mortality and morbidity was conducted in Finland. The material consisted of 1092 employees from two mines, 248 of whom were deceased. Among the causes of death there was found an excess due to lung cancer and asbestosis but not due to cancers of the digestive system, and there were no confirmed mesotheliomas in either cases or controls sampled from the neighbourhood of the mines. Among the asbestos workers there were more heavy smokers than among the controls. A three-fold risk of dyspnoea and a two-fold risk of cough were estimated for the asbestos workers as compared with the controls, after adjusting for smoking. The effects of asbestos seen in this study are likely to appear more modest than those reported in most other studies. The main reason for this difference is assumed to be the type of reference population and the relatively short duration of exposure.

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Cancer among workers in the asbestos textile industry

MURIEL L. NEWHOUSE [1]

STUDIES IN THE UNITED STATES

Reports of studies relating specifically to asbestos textile workers are relatively uncommon. In the United States, however, two important cohort studies have been undertaken. Enterline (Enterline & Kendrick, 1967) refined his previous study (Enterline, 1965) in order to contrast the experience of three different types of asbestos worker (those producing building products, friction materials and textile goods) with cotton textile workers. All asbestos workers had a higher mortality than did cotton workers, but the textile workers had the most severe experience. For cancer of the respiratory system among the three groups of asbestos workers the standardised mortality ratios (SMR) were 130, 120 and 229. Physical inspection of the plants showed the textile factories to be the most dusty.

Mancuso & El-Attar (1967) have also followed a cohort of persons who were employed at an asbestos plant in 1938 and 1939. The 1939 cohort had a lower mortality experience than did the 1938 cohort, which provided an indication of the effectiveness of environmental control in subsequent years. Nevertheless, both cohorts had a severe mortality experience from respiratory and gastro-intestinal cancer. Among the 1265 males, there were eight deaths from peritoneal mesothelioma; and among the 228 females there was one.

STUDIES IN THE UNITED KINGDOM

Knox *et al.* (1968) have also discussed mortality experience at an asbestos textile factory. They showed that, where the provisions of the Asbestos Industry Regulations, 1931 had been applied, the observed incidence of bronchogenic carcinoma in men exposed for 20 or more years without any exposure prior to 1933 was no greater than that expected for the general population. Four mesothelial tumours had been diagnosed since 1963. Eighteen of these tumours are now known to have occurred in past workers, five of whom were first employed after 1931. The factory, though a major user of chrysotile asbestos, had used a certain amount of crocidolite in various processes up until 1969 (Lewinsohn [2]).

In another English factory with a large labour force, four workers certified as suffering from "asbestosis" have died of mesothelial tumours. Three of these were exposed to both crocidolite and chrysotile asbestos, and one, so far as is known, to chrysotile alone. This factory only used crocidolite between 1941 and 1951 (Beverley [3]). Mortality data over the next decade should give valuable information.

The mortality of workers at a London factory has been studied by us in detail (Newhouse, 1969; Newhouse & Wagner, 1969; Newhouse *et al.*, 1972). Until the mid-1950's, this factory had a large textile department, and it also produced sectional asbestos piping, some friction material and other goods. It used large amounts of crocidolite, but amosite and chrysotile were also used. Factory records provided details of dates and duration of employment as well as of the nature of jobs. A cohort of males employed since the implementation of the Asbestos Regulations was followed until March 1970, and a cohort of women first employed between 1936 and 1942 was followed until December 1968. Tracing

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of these two populations has been done through national records (Newhouse & Williams, 1967), and copies of death certificates have been provided by the Office of Population Censuses and Surveys. Where possible, death certificate information has been supplemented by autopsy reports and the examination of histological specimens. The degree of dust exposure was evaluated subjectively: those employed outside production departments, or working where there were low proportions of asbestos in the product, were judged to have low to moderate exposure; those involved in the opening and preparing of fibre, manufacture of textiles and other products were said to have severe exposure.

The mortality experienced by the workers has been assessed by comparing the number of observed deaths with the number of expected deaths in the population of England and Wales. The expected deaths were calculated by the man-years method (Case & Lea, 1955), multiplying years of risk by death rates. Table 1 shows the mortality of male workers with low to moderate exposure and less than 2 years' exposure, and Table 2 those with more than 2 years of work at the factory.

In general, the workers with minor exposure and short periods of employment show no excess mortality; but among those with long periods of employment significant excess mortality from cancer of the lung and from other cancers is now beginning to show, 25 years after first employment. During the first 10 years of follow-up, no excess mortality was shown by any group, and data relating to this period are not shown in Tables 1–4. The maximum period of follow-up for males was 37 years.

Among those with severe but short periods of employment (Table 3), there is a significant excess of deaths from lung cancer (ICD 162, 163)[1], which is first seen after 15 years of follow-up, and highly significant excesses of other cancers after 20 and 25 years. The effect is much more marked in those who worked for longer than 2 years at the factory (Table 4).

There were only 126 women with low to moderate exposure; there was, however, a statistically signifi-

[1] International Classification of Diseases and Causes of Death, 7th Revision, World Health Organization, Geneva, 1957.

Table 1. Mortality of male workers. Low–moderate exposure. Less than 2 years in job

Follow-up years No. of men	10– 734		15– 583		20– 365		25+ 179	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
All causes	29	22.2	15	23.0	13	16.2	13	13.4
Cancer of lung (162, 163)[a]	1	1.8	1	1.9	1	1.2	3	1.2
Other cancers	8[b]	3.1	2	3.1	1	2.0	1	1.8
Respiratory disease, excluding cancer	6	3.3	3	3.4	2	2.5	2	2.0
Other unspecified	14	14.0	9	14.6	9	10.5	7	8.4
Pleural mesothelioma	0		0		0		1	
Peritoneal mesothelioma	0		0		1		0	

[a] ICD ratings.
[b] $P < 0.05$.

Table 2. Mortality of male workers. Low–moderate exposure. More than 2 years in job

Follow-up years No. of men	10– 479		15– 374		20– 228		25+ 97	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
All causes	17	19.1	15	18.4	13	9.6	16[b]	7.8
Cancer of lung (162, 163)	3	1.6	2	1.6	2	0.9	3[a]	0.7
Other cancers	2	2.6	1	2.6	1	1.3	8[c]	1.0
Respiratory disease, excluding cancer	1	2.9	3	2.8	2	1.4	3	1.2
Other unspecified	11	11.9	9	11.5	8	5.9	2	4.9
Pleural mesothelioma	0		0		0		2	
Peritoneal mesothelioma	0		0		1		2	

[a] $P < 0.05$. [b] $P < 0.01$. [c] $P < 0.001$.

Table 3. Mortality of male workers with severe exposure to asbestos dust. Less than 2 years in job

Follow-up years No. of men	10– 845		15– 708		20– 540		25+ 324	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
All causes	23	17.6	25	18.3	25[a]	13.0	32[b]	18.5
Cancer of lung (162, 163)	2	1.5	7[a]	1.8	3	1.3	8[a]	2.0
Other cancers	1	2.4	4	2.6	8[b]	1.9	6	2.7
Respiratory disease, excluding cancer	3	2.8	2	2.5	2	1.7	5	2.4
Other unspecified	17	10.9	12	11.4	12	8.1	13	11.4
Pleural mesothelioma	0		1		0		3	
Peritoneal mesothelioma	0		1		1		2	

[a] $P < 0.01$. [b] $P < 0.001$.

Table 4. Mortality of male workers with severe exposure to asbestos dust. More than 2 years in job

Follow-up years No. of men	10– 482		15– 414		20– 281		25+ 127	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
All causes	18	22.5	40[b]	21.9	32[b]	13.8	24[a]	12.6
Cancer of lung (162, 163)	3	2.1	16[b]	2.0	10[b]	1.2	7[a]	1.1
Other cancers	5	3.2	6	3.1	4	1.8	8[b]	1.7
Respiratory disease, excluding cancer	4	3.6	6	3.3	7[a]	2.1	3	1.9
Other unspecified	6	13.7	12	13.5	11	8.7	6	7.9
Pleural mesothelioma	0		1		1		0	
Peritoneal mesothelioma	0		2		3		2	

[a] $P < 0.07$. [b] $P < 0.007$.

Table 5. Mortality of women workers with severe exposure to asbestos dust

No. of women	Less than 2 years in job 557		More than 2 years in job 239	
	Obs.	Exp.	Obs.	Exp.
All causes	55	49.9	56[b]	24.5
Cancer of lung and pleura (162, 163)	6[b]	1.0	14[b]	0.5
Other cancers	16	12.4	17[b]	6.1
Respiratory disease, excluding cancer	10	7.4	11[a]	3.6
Other unspecified	23	29.1	14	14.3
Pleural mesothelioma	3		3	
Peritoneal mesothelioma	3		2	

[a] $P < 0.01$. [b] $P < 0.001$.

cant excess of deaths from lung cancer in this group (2 observed: 0.3 expected; $P<0.05$). The experience of the 796 severely exposed women (Table 5), a high proportion of whom worked in textile departments, is similar to that of the men. The maximum follow-up period for this group was 33 years; and 11 deaths from mesothelial tumours were found among them.

Cancer of lung

Bronchogenic lung cancer is the most frequent single cause of death from cancer in this group of workers. Among the 2640 males followed for more than 10 years since first employment at the factory, there were 72 deaths from lung cancer, including 9 deaths from pleural mesothelioma; and 67 deaths from other cancers, including 15 peritoneal mesothelioma.

The smoking habits of over 1300 of the male and 480 of the female asbestos factory workers have been studied, and their mortality from lung cancer over a 10-year period has been examined (Berry *et al.*, 1972). The number of deaths has been compared with the number of expected deaths in the general population

Table 6. Comparison of observed and expected cancers of lung under the two hypotheses[a]

Smoking habits	Observed (adjusted)	Expected (non-occupational)	Expected (occupational and non-occupational)	
			Additive	Multiplicative
Men:				
Never smoked	0	0.0	0.9	0.1
Ex-smokers	1.6	0.2	1.1	0.6
Smokers	25.5	9.9	25.1	26.4
Women:				
Never smoked	1.7	0.2	4.7	1.9
Smokers	15.5	1.4	12.5	15.3

[a] This table first appeared in *The Lancet*, ii, 476–479, 1972, and is reproduced here by kind permission of the editor of that journal.

of the area. By adjusting the expected figures to allow for smoking habits, the influence of the occupational factor could be studied (Table 6). No significant excess was found in workers, whether smokers or non-smokers, with low to moderate exposure; but among workers who smoked and who were severely exposed, the excess was highly significant. The analysis, particularly of the data relating to the women among whom there was a high proportion of non-smokers, supports the multiplicative hypothesis for the action of two carcinogens.

Mesothelial tumours

There were 35 deaths from mesothelial tumours among the men and women workers in the cohort, and a further eight among laggers employed by the firm on contract. By relating the number of tumours to the number of subject years experienced by members of the cohort, the length of employment and the degree of exposure to asbestos dust (Table 7), it can be seen that the mesothelioma rate/100,000 subject years increases both with the severity and with the length of exposure. Laggers have a higher rate than do factory production workers.

There is histological verification of this group of 43 tumours, and of a further 14 which occurred among men first employed before the regulations of 1931 and who are not therefore included in the cohort analysis. In five of the pleural tumours, the death certificate gives the cause of death as bronchial carcinoma or lung cancer, and in two as asbestosis. Among the peritoneal tumours, the cause of death was given in five as carcinomatosis, in three as carcinoma of the pancreas, in two as carcinoma of the rectum, and in one as fibrosarcoma of the diaphragm. The accuracy of certification appears to have improved in the past 5 years.

Gastro-intestinal tumours (ICD 150–157)

Among the males, there was a significant excess of deaths coded to the above rubrics (31 observed: 20.35 expected; $P<0.05$). Histological cover of this group was poor, specimens for examination could be obtained from only eight of the cases; in three of these the cause of death was revised to peritoneal mesothelioma. Among the women there was no significant excess of deaths from this cause (11 observed: 7.3 expected; $P=0.12$).

Risk from individual fibre type

In this factory most workers had been in jobs where crocidolite was used, but most commonly together with chrysotile and amosite asbestos. Very few had been exposed to one type of fibre alone. It was not possible from the information available to

Table 7. Mesothelioma rate/100,000 subject years after more than 10 years' follow-up

Length of employment		Exposure								
		Low–Moderate			Severe			Laggers		
		n	s/y	rate	n	s/y	rate	n	s/y	rate
Males	less than 2 years	2	7899	25	8	11,193	71	4	2550	157
	more than 2 years	5	4926	102	9	5,851	154	4	1530	261
Females	less than 2 years	0	931	—	6	7,176	84	—	—	—
	more than 2 years	0	640	—	5	3,538	141	—	—	—

Table 8. Excess annual death rates for cancer among male workers per 100,000 subject years after more than 10 years' follow up

Length of employment	Exposure			
	Low–moderate		Severe	
	Lung cancer	Other cancer	Lung cancer	Other cancer
Less than 2 years	−2	25	119	84
More than 2 years	105	92	506	227

relate the cancer risk to exposure to any particular fibre.

Excess annual death rates

These rates were calculated in order to estimate the order of risk to which workers in this factory were exposed (Table 8). They were obtained by relating the difference in the observed and expected number of deaths to the number of years at risk.

Clearly the highest risk, both for cancers of the lung and pleura and for other cancers, is among those with severe exposure and long periods of employment. For lung cancers there is an excess annual death rate of more than 500, and for other cancers more than 200.

Future areas of research

Two outstanding problems remain. Firstly, the relative carcinogenicity of different asbestos fibres should be established. Evidence implicates crocidolite, and there are now stringent regulations governing its use in the UK, but not in other countries. Selikoff *et al* (1971) have shown that workers exposed only to amosite asbestos have a significant excess of deaths from lung cancer, and that among 230 workers five died of mesothelial tumours. Among those exposed only to chrysotile asbestos, deaths from these tumours are also not uncommon. The studies reported above strongly suggest a dose relationship in the production of tumours. Studies of single fibre exposures, where systematic measurement of dust levels have been undertaken, should clarify both these questions.

Secondly, there remains doubt as to whether mortality from gastro-intestinal tumours is a specific risk to asbestos workers. Selikoff *et al.* (1968) and Elmes & Wade (1965) have found excess mortality from cancers of the stomach, colon and rectum in groups of workers studied. McDonald *et al.* (1970) found a higher mortality from these causes in heavily exposed workers. Histological verification of the presence of these tumours is incomplete, since autopsy is not commonly performed unless asbestosis or mesothelioma is the suspected cause of death. Cancer registers might clarify this point by careful recording of occupational histories.

ACKNOWLEDGEMENTS

I would like to acknowledge my statistical colleagues, Mr David Hill, Dr Peter Oldham and Mr Geoffrey Berry, who prepared the computer programmes and the statistical analyses on which this paper is based.

SUMMARY

Studies in the United States show that asbestos textile workers are heavily exposed to asbestos dust. In studies on mortality in a London factory a high mortality was found from bronchogenic carcinoma and mesothelial tumours. Smoking and asbestos exposure appeared to have multiplicative effects on the occurrence of lung cancers. Both mesothelial tumours and lung cancers appeared to show a dose-response relationship to length of exposure and degree of exposure to dust. Due to mixed dust exposures no conclusions could be drawn as to the relative carcinogenicity of any particular asbestos fibre. Evidence concerning a specific risk of gastro-intestinal tumours in this group of workers was inconclusive. Studies of mortality combined with measurements of asbestos in the environment might confirm the dose-response relationship and histological studies of autopsy or biopsy material from asbestos workers would clarify the question of the risk of gastro-intestinal cancer.

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Cancer risk of insulation workers in the United States[1]

I. J. SELIKOFF, E. C. HAMMOND & H. SEIDMAN

In 1963, information became available which indicated that asbestos insulation workers employed in the construction industry were subject to a significant cancer hazard associated with their work (Selikoff *et al.*, 1964). Studying the mortality experience of the 632 members on the rolls of the New York–New Jersey branches of the insulation workers' union on 1 January 1943, for the 20-year period ending 31 December 1962, it was found that death from cancer was almost three times as frequent as expected. The death rate from cancer of the bronchus and pleura was 6.8 times as high as that for the general US white male population, both age and date being taken into consideration; and cancer of stomach, colon and rectum was found to be three times as common. Attention was called to the occurrence of pleural and peritoneal mesotheliomas (Selikoff *et al.*, 1965a).

These studies were subsequently extended to investigate the interrelationships between asbestos exposure and cigarette smoking in the production of lung cancer, the significance of mesothelioma (Selikoff *et al.*, 1970) and to obtain further evidence on the possible relationship between asbestos exposure and the occurrence of gastro-intestinal cancer.

The data were insufficient, however, to resolve a number of questions which are important for a full evaluation of the problem and to provide guidance for the establishment of needed administrative and industrial control measures. To obtain such additional information, we have continued and widened our investigations.

CURRENT STUDIES

Using approaches and methods previously reported (Selikoff *et al.*, 1964; Selikoff *et al.*, 1965b), we have studied the experiences of three cohorts of workers exposed to asbestos insulation materials.

1. *New York–New Jersey cohort.* We have continued our surveillance of the New York–New Jersey insulation workers. By 31 December 1962, 262 had died; thus, 370 were still alive on 1 January 1963. Each has been followed up until 31 December 1971.

Two additional groups of workers have been investigated:

2. In one study, we registered *the entire membership of the insulation workers union in the United States and Canada*[2] on 1 January 1967 (17,800; including members of the New York–New Jersey locals mentioned above), and recorded many characteristics for each man including date of birth and onset of insulation work. Each man has since been observed until 31 December 1971.

3. A third group consisted of *the entire workforce (1941–45) of a factory in an eastern US city* which manufactured amosite asbestos insulation materials (including insulating block and pipe covering, and asbestos mattresses), for use by insulation workers in the construction industry, especially in ship construction and repair. The plant opened its doors in June 1941 and remained in business until November 1954. Very few of the production workers studied by us had had any prior occupational exposure to asbestos. Between 1941 and the end of 1945 a total of 933 men were hired; of these, some worked for as little as one day, while others worked until 1954 when the plant closed. Results of physiological studies of men in this plant have been reported (Bader *et al.*, 1961). Recently, we have traced the entire group up until 31 December 1971 in order to evaluate the cancer risk among them. Eight hundred and seventy-

[1] From the Environmental Cancer Research Project of the American Cancer Society and the Mount Sinai School of Medicine of the City University of New York.

[2] International Association of Heat and Frost Insulators and Asbestos Workers, AFL–CIO.

Table 1[a]. Expected[b] and observed number of deaths among 623 New York–New Jersey asbestos insulation workers 1 January 1943–31 December 1971, twenty or more years after onset of first exposure to asbestos

	1943–51		1952–61		1962–71		Total, 1943–71	
	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed
Total cancer: all sites	11.3	28	18.3	57	17.6	104	47.2	189
Lung cancer	1.5	13	3.5	23	5.1	48	10.1	84
Pleural mesothelioma	n.a.[c]	1	n.a.	2	n.a.	5	n.a.	8
Peritoneal mesothelioma	n.a.	1	n.a.	3	n.a.	20	n.a.	24
Cancer of stomach, colon, rectum, oesophagus	4.1	7	5.0	18	3.9	16	13.0	41
Cancer all other sites	5.7	6	9.8	11	8.6	15	24.1	32
Asbestosis	n.a.	1	n.a.	10	n.a.	22	n.a.	33
All other causes	65.1	44	89.4	94	78.3	61	232.8	199
Total all causes	76.4	73	107.7	161	95.9	187	280.0	421
Person-years of observation	3726		4406		2898		11,030	

[a] 632 members were on the union's rolls on 1 January 1943. Nine died before reaching 20 years from first employment. All others entered these calculations upon reaching the 20-year from onset of first exposure point.
[b] Expected rates are based upon age-specific death rate data of the US National Office of Vital Statistics from 1949–67. Rates were extrapolated 1943–48 from rates for 1949–55, and for 1968–71 from rates for 1961–67.
[c] US death rates not available, but these are rare causes of death in the general population.

seven (94%) were fully traced, and the remainder were partially traced for varying periods. We have analysed their mortality experience in relation to the duration of employment, age at onset of employment and lapsed period from onset of employment.

FINDINGS IN INSULATION WORKERS IN THE CONSTRUCTION INDUSTRY

The experiences of the two cohorts of insulation workers in the construction industry (New York–New Jersey and US–Canada cohorts) amply confirm the cancer risk first identified in 1963. By 31 December 1971, 430 of the original 632 (New York–New Jersey cohort) had died, 421 after reaching 20 years from onset of employment. One hundred and eighty-nine (45% of the 421 total deaths) died of cancer, whereas 47.2 such deaths had been expected (Table 1).

The average age of the insulation workers throughout the United States and Canada (US–Canada cohort) was much lower, and they therefore had considerably less work experience than that of the New York–New Jersey cohort (Hammond & Selikoff [1]). Nevertheless, the excess of cancer deaths was very much the same, these deaths occurring among the older men who had longer work experience. Relatively few deaths were found among the younger men, despite the large number in the cohort. 144.09 cancer deaths were expected and 459 occurred, representing 41% of the total of 1092 deaths observed in the period 1967–71 (Table 2).

Lung cancer

Bronchogenic carcinoma was found to be a leading cause of death among asbestos insulation workers, accounting for approximately 45% of fatal neoplasms. In the first two series reported, it was responsible for 20% of all deaths and was almost three times as common as were pleural and peritoneal mesothelioma. Recent histological studies of tumours from these patients show the distribution of cell types to be that of lung cancer found in the general population (Kannerstein & Churg, 1972), and our clinical experience indicates that their prognosis was equally poor. These findings stress the urgency of preventive measures, such as dust control and the avoidance of cigarette smoking (Selikoff *et al.*, 1968; Hammond & Selikoff[1]).

Gastro-intestinal cancer

In our initial reports (Selikoff *et al.*, 1964; Hammond *et al.*, 1965) attention was called to the then unexpected finding of a moderate excess of gastro-intestinal cancer among New York insulation workers. Nevertheless, relatively few deaths were studied and firm conclusions were not considered warranted. We have now collected additional data, and these give the same indication very much at the

[1] See p. 312 of this publication.

Table 2. Expected[a] and observed deaths among 17,800 asbestos insulation workers in the United States and Canada 1 January 1967–31 December 1971

	Total		Distribution by duration from onset of exposure			
			Less than 20 years		20 years and more	
	Expected	Observed	Expected	Observed	Expected	Observed
Total deaths	805.63	1092	178.94	211	626.69	881
Cancer: all sites	144.09	459	26.31	51	117.78	408
Lung cancer	44.42	213	7.03	22	37.39	191
Pleural mesothelioma	n.a.[b]	26	n.a.	2	n.a.	24
Peritoneal mesothelioma	n.a.	51	n.a.	3	n.a.	48
Cancer of stomach	6.62	16	0.97	1	5.65	15
Cancer of colon, rectum	17.51	26	2.51	3	15.00	23
Cancer of oesophagus	3.21	13	0.44	1	2.77	12
All other cancers	72.33	114	15.36	19	56.97	95
Asbestosis	n.a.	78	n.a.	5	n.a.	73
All other causes	661.54	555	152.63	155	508.91	400
Number of men	17,800		12,681		5,119	
Person-years of observation	86,300		62,673		23,627	

[a] Expected deaths are based upon age-specific death rate data of the US National Office of Vital Statistics. Rates for 1968–71 were extrapolated from rates for 1961–67.
[b] US death rates not available, but these are rare causes of death in the general population.

same level of excess, i.e., at two or three times expected deaths (Tables 1 & 2). These findings were made for cancer of the oesophagus, stomach, and of the colon and rectum. A similar excess has been found among insulation workers in Belfast (Elmes & Simpson, 1971). Taken together, these experiences suggest that the increase is real. While they are not responsible for a major proportion of excess deaths (lung cancer, mesothelioma and asbestosis account for more), the incidence of gastro-intestinal cancer is nevertheless substantial: in the New York–New Jersey group 13.0 deaths were expected and 41 occurred; and in the US–Canada survey, 27.3 were expected and 55 occurred. Moreover the finding may be of considerable theoretical importance, in view of the dearth of useful hypotheses concerning the etiology of gastro-intestinal cancer in general, and of cancer of the colon and rectum in particular.

Other neoplasms

The knowledge that a number of tissues are subject to asbestos-induced cancer, coupled with the observation that fibres and fibrils may be found in many organs, admits the possibility that a neoplastic effect exists for sites other than those heretofore identified. Examination of this hypothesis is hampered by the infrequency of some such tumours in general and by the as yet inadequate number of observations available. We do not at present find it useful, therefore, to comment on this question, although we have been particularly interested in cancer of pancreas, brain and oro-pharynx, in genito-urinary cancer, and lymphoma and leukaemia.

RISK WITH FACTORY EXPOSURE TO ASBESTOS INSULATION MATERIALS

In 1964, Smith (1965) called attention to the multiplicity of materials to which insulation workers were exposed. It seemed justified to seek evidence on whether asbestos insulation materials *per se* were specifically related to various diseases. If not, investigation of other insulation materials would be required. We therefore studied the work force of the insulation materials factory noted above, in which exposure was only to asbestos insulation, and not to other insulation materials used in the construction industry (e.g., fibrous glass, calcined diatomaceous earth, rock wool, etc.)

Four hundred and eighty-four deaths are known to have occurred among the 877 men fully traced up to 31 December 1971. The distribution of deaths strongly resembled that seen among insulation workers who install such materials, despite the fact that observation of this cohort has reached only 25–30 years from onset. Thus, lung cancer accounted for 73 deaths, although only 11.41 were expected; and gastro-intestinal cancer for 26, with 12.86 expected

Table 3. Expected[a] and observed deaths among 933[b] amosite asbestos factory workers first employed 1941–45, and observed to 31 December 1971

	Before 1952		1952–61		1962–71		Total, 1941–71	
	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed
Total deaths	69.98	88	108.27	146	121.24	250	299.49	484
Total cancer: all sites	9.81	15	18.72	44	21.63	84	50.16	143
Lung cancer	1.49	3	3.71	23	6.21	47	11.41	73
Pleural mesothelioma	n.a.[c]	1	n.a.	0	n.a.	2	n.a.	3
Peritoneal mesothelioma	n.a.	0	n.a.	0	n.a.	4	n.a.	4
Cancer of stomach	1.45	3	1.84	4	1.29	4	4.58	11
Cancer of colon, rectum	1.56	2	2.61	5	2.88	8	7.05	15
Cancer of oesophagus	0.27	0	0.45	0	0.51	0	1.23	0
All other cancers	5.04	6	10.11	12	10.74	19	25.89	37
Asbestosis	n.a.	3	n.a.	7	n.a.	17	n.a.	27
All other causes	60.17	70	89.55	95	99.61	149	249.33	314

[a] Expected rates are based upon age-specific death rate data of the US National Office of Vital Statistics from 1949–67. Rates were extrapolated for 1941–48 from rates for 1949–55, and for 1968–71 from rates for 1961–67.
[b] 933 men were employed. In 5 cases, ages were not known and these men have been excluded from these calculations. 877 men were traced to death or to 31 December 1971. 51 men were partially traced and remain in the calculations until lost to observation.
[c] US death rates not available, but these are rare causes of death in the general population.

(including oesophagus). Mesothelioma caused seven deaths, and asbestosis 27 (Table 3).

These data indicate that asbestos insulation materials are capable of causing the disease patterns seen among insulation workers; there is no need to invoke the influence of other insulation materials.

EPIDEMIOLOGIC VARIABLES

Lapsed period from onset of exposure

It has long been inferred, on the basis of rather limited data, that asbestos cancer largely occurs more than twenty years from onset of exposure.

Table 4. Deaths from lung cancer and pleural mesothelioma among 17,800 asbestos insulation workers in the US and Canada, 1 January 1967–31 December 1971: relation to elapsed period from onset of work exposure

Years from onset	Lung cancer			Pleural mesothelioma
	Expected deaths[a]	Observed deaths	Ratio	Observed deaths
<10	0.48	0	—	0
10–14	1.69	4	2.4	0
15–19	4.86	18	3.7	2
20–24	7.55	25	3.3	4
25–29	8.50	41	4.8	7
30–34	6.24	44	7.1	4
35–39	3.53	23	6.5	1
40–44	4.04	24	5.9	3
45–49	3.72	17	4.6	4
50+	3.81	17	4.5	1
Total	44.42	213	4.8	26

[a] Expected deaths are based upon age-specific death rate data of the US National Office of Vital Statistics. Rates for 1968–71 were extrapolated from data for 1961–67.

Experiences in the cohorts studied indicate that lung cancer may occur in considerable excess as early as 10–14 years from onset, with 0.65 expected deaths *versus* 10 observed among those working in the amosite factory for more than one year (Table 5b). Moreover, death rates for lung cancer were significantly increased in the US–Canada insulation workers cohort 15–19 years from onset (4.86 deaths were expected and 18 occurred) (Table 4).

On the other hand, in terms of numbers, most deaths from lung cancer occurred among insulation workers 30–39 years from onset of exposure, or later. Therefore, it would be difficult to evaluate fully the effects of asbestos exposure unless observation was possible for at least 40 years from the onset of work exposure.

Age at onset

A number of methodological difficulties are encountered in studying the effect of age at onset on lung cancer rates. Not the least are the secular changes in cigarette smoking habits in the past 50 years (Hammond, 1966), and the concurrent increases in lung cancer death rates in the general population, which must be taken into account.

With these in mind, there seem to be no striking variations in the incidence of lung cancer as a result of differences in age at which asbestos work exposure first began (Table 6).

Duration of exposure

In one cohort, it was possible to study the effect of differences in length of exposure. While all the in-

Table 5a. Age at onset of employment, 1941–45, of 933 workers in an amosite asbestos insulation factory

Age at employment	Total	Duration of employment		
		< 3 months	3–11 months	1 + years
15–19	94[a]	42	30	21
20–24	114	35	34	45
25–29	120	28	48	44
30–34	114	32	40	42
35–39	106	27	40	39
40–44	101	30	38	33
45–49	83	29	21	33
50–54	90	26	27	37
55–59	55	14	21	20
60–64	30	6	12	12
65–69	16	6	4	6
70–74	4	1	3	—
75–79	1	—	—	1
Unknown	5	2	3	—
Total	933[a]	278	321	333

[a] Includes one man with unknown length of employment.

Table 5b. Lung cancer among 326 amosite asbestos workers employed for 1 year or more starting 1941–45, and observed to 31 December 1971.[a] Expected and observed deaths [b]

Attained years from first employment	Attained ages													
	Total		< 40		40–49		50–59		60–69		70–79		80–89	
	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
1–9	0.68	2	0.02	0	0.09	0	0.30	2	0.22	0	0.05	0	—	—
10–14	0.65	10	0.02	0	0.07	0	0.19	4	0.29	5	0.08	1	—	—
15–19	0.72	11	0.01	0	0.07	0	0.20	3	0.26	4	0.17	4	0.01	0
20–24	1.03	8	—	—	0.10	2	0.29	5	0.39	1	0.22	0	0.03	0
25–29	1.01	14	—	—	0.06	1	0.27	4	0.37	7	0.26	6	0.05	0
	4.09	45	0.05	0	0.39	3	1.25	18	1.53	17	0.78	11	0.09	0

[a] Seven were omitted, four because of prior occupational exposure to asbestos and three who had not been traced after the first year. Five were partially traced and remained in the calculations only to the date of last observation. All others entered the calculations after first year from onset.

[b] Expected rates are based upon age-specific death rate data of the US National Office of Vital Statistics from 1949–67. Rates were extrapolated for 1941–48 from rates for 1949–55, and for 1968–71 from rates for 1961–67.

Table 6. Expected and observed deaths of lung cancer among 17,800 asbestos insulation workers, 1 January 1967–31 December 1971; distribution by age at onset of exposure

Age at onset of exposure	Deaths of lung cancer		
	Expected[a]	Observed	Ratio
< 25	18.19	102	5.61
25–34	15.20	66	4.34
35–44	8.78	38	4.33
45 +	2.25	7	3.11

[a] Expected deaths are based upon age-specific death rate data of the US National Office of Vital Statistics. Rates for 1968–71 were extrapolated from rates for 1961–67.

sulation materials factory employees started work between 1941 and 1945, approximately one-third of these worked for less than three months, another third for three to eleven months, and the remaining third for a year or more (Table 5a). All worked in the same factory and had similar exposure to the same asbestos starting at the same point in time. The only significant variable was duration of exposure. All were traced to the end of 1971.

Our findings indicate that duration of exposure, and thus presumably total dose, has an important influence on lung cancer rates. Those with less

Table 7. Expected and observed deaths of lung cancer among 876 amosite asbestos factory workers, first employed 1941–45, and observed to 31 December 1971.[a] Distribution by duration of employment

Duration of employment	Number of men	Person-years of observation	Deaths of lung cancer		
			Expected[b]	Observed	Ratio
< 3 months	256	5,869	3.55	13	3.66
3–11 months	294	6,158	3.58	15	4.19
1 + years	326	6,912	4.09	45	11.00
Total	876	18,939	11.22	73	6.51

[a] This table excludes 57 men. Ten died during first year of employment, 39 could not be traced after the first year, 7 had prior occupational exposure to asbestos and 1 had employment of uncertain duration. Seventeen men of the 876 were partially traced and remained in the calculations until lost to observation.

[b] Expected rates are based upon age-specific death rate data of the US National Office of Vital Statistics, 1949–67. Rates were extrapolated for 1941–48 from rates for 1949–55, and for 1968–71 from rates for 1961–67.

Table 8. Mortality experience of 337 asbestos insulation workers 1 January 1963–31 December 1971, analysed by duration of shipyard employment

Cause of death	Shipyard employment					
	None (98)		Up to two years (118)		Three or more years (121)	
	Observed	Expected[a]	Observed	Expected	Observed	Expected
Total cancer: all sites	25	3.8	28	4.8	29	5.8
Lung cancer	11	1.2	9	1.4	17	1.6
Pleural mesothelioma	2	n.a.[b]	2	n.a.	0	n.a.
Peritoneal mesothelioma	6	n.a.	6	n.a.	4	n.a.
Cancer of stomach, colon, rectum, oesophagus	3	0.8	2	1.0	5	1.3
Cancer all other sites	3	1.8	9	2.4	2	2.9
Asbestosis	4	n.a.	11	n.a.	5	n.a.
All other causes	8	16.2	18	20.3	21	26.6
Total deaths	37	20.0	57	25.1	55	32.4

[a] Expected deaths are based upon age-specific death rate data of the US National Office of Vital Statistics. Rates for 1968–1971 were extrapolated from rates for 1961–1967.

[b] US death rates not available, but these are rare causes of death in the general population.

Table 9. Expected[a] and observed deaths among asbestos insulation workers in shipyard and construction industry work, 1 January 1967–31 December 1971

	Workmen registered in union locals in south-west, mid-west and central states		Workmen registered in shipyard locals	
	Expected	Observed	Expected	Observed
Total deaths	316.28	446	39.56	34
Total cancer: all sites	56.34	172	7.91	16
Lung cancer	17.47	79	2.67	9
Pleural mesothelioma	n.a.[b]	10	n.a.	2
Peritoneal mesothelioma	n.a.	13	n.a.	1
Cancer of stomach	2.56	6	0.36	0
Cancer of colon, rectum	6.75	12	0.98	0
Cancer of oesophagus	1.26	6	0.20	1
All other cancers	28.30	46	3.70	3
Asbestosis	n.a.	26	n.a.	3
All other causes	259.94	248	31.65	15
Number of men	7,289		462	
Person-years of observation	35,310		2,244	

[a] Expected deaths are based upon age-specific death rate data of the US National Office of Vital Statistics. Rates for 1968–71 were extrapolated from rates for 1961–67.

[b] US death rates not available, but these are rare causes of death in the general population.

than three months of work showed a definite increase in lung cancer risk (13 observed *versus* 3.55 expected), and those with three to eleven months' exposure much the same increase; while workers employed for one year or over had a much more substantial increase (45 observed, 4.09 expected) (Table 7).

Fibre type

Crocidolite was not used for insulation work in the United States during the period covered by our study. Indeed, very little crocidolite was imported into the United States until after the Second World War (Selikoff *et al.*, 1970). Thus, exposure to this fibre cannot explain the cancer risk observed.

Both chrysotile and amosite have been used in insulation materials in the US, the former since the turn of the century (Selikoff *et al.*, 1965b) and the latter since the mid-1930's (Selikoff *et al.*, 1972). Amosite has been used principally in ship insulation and chrysotile in general construction and industrial work, although it is also found in some shipyard materials.

It is difficult to compare the relative disease potential of the two varieties of fibres in insulation work, since cohorts which differ in this respect may also differ in other respects, including type and intensity of exposure, duration of exposure and lapsed period from onset of exposure. Nevertheless, two sets of observations are available which may be of interest. We have compared the mortality experience of insulation workers in the New York–New Jersey group according to their duration of shipyard employment (ship insulation being fairly important in the New York harbour area, especially between 1939 and 1945). Of the 370 men in the union on 1 January 1963 who were survivors of the 1943 cohort, we were able to obtain histories of shipyard employment by personal interview from 337. The results indicate that the mortality experience in men with shipyard employment, with its greater potential for amosite exposure, did not differ substantially from that of men in the same union, at the same period of time, without such employment (Table 8).

In the US–Canada survey, each union member was registered in a specific union local, several of which were engaged entirely in shipyard work. Others, in the central and mid-western parts of the country, had no such experience. This is not to say that individual members of these locals did not, in the past, do some shipyard work, but it is unlikely to have constituted a major part of their work-history.

We have analysed the mortality experience of men in the two groups of locals between 1967 and 1971. There does not seem to have been any significant difference between them (Table 9). At least as reflected in these two sets of data, construction industry insulation work, with its preponderance of chrysotile exposure, was no more or less hazardous than was shipyard work, with its wider use of amosite materials.

SUMMARY

A serious cancer risk has been demonstrated among asbestos insulation workers in the United States. Approximately one death in five, an extraordinary incidence, has been the result of lung cancer. Gastro-intestinal cancer was more than doubled in incidence, and mesothelioma was responsible for 7% of all deaths.

An increased incidence of lung cancer was seen as little as 10–14 years from the onset of work exposure; we suspect that this tends to be associated with more intense exposure, as among factory workers. However, the greater increase occurred 30–45 years from onset, especially among the less intensely exposed insulation workers. It would be difficult to evaluate fully the effects of asbestos exposure without observation for at least 40 years from onset of exposure.

Data have been presented indicating that these risks are associated with the asbestos insulation materials *per se*.

We were not able to find evidence that the use of chrysotile in insulation work was associated with a greater risk than was amosite, or *vice versa*. We have no knowledge of the comparative effect which crocidolite might have; it was not used in US insulation work.

ACKNOWLEDGEMENTS

We wish to express our indebtedness to the skilful assistance of our staffs. We are particularly grateful to Mrs Janet S. Kaffenburgh, Mrs Selma Annenberg, Mrs Frances Perez, Mrs Dorothy Perron, Mrs Shirley Levine, Mrs Rayla Margoles and Mr Charles V. Nolan for the successful tracing of the subjects in these epidemiological

studies, and to Mr Lawrence Garfinkel and Mrs Doris Fleisher for their devoted help in many aspects of the work.

This work was supported in part by US Department of Health, Education and Welfare research grants OH 00320 and ES 00358, and by research grant U 1272 of the Health Research Council of the City of New York, to the Mount Sinai School of Medicine.

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Cancer in relation to environmental exposure

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There are a number of difficulties involved in making a report on environmental asbestos-induced malignant tumours:

(1) Firstly, the term "environmental cancers" has not yet been clearly defined. With regard to asbestos, the literature lists as "environmental cancers" those which occurred in a number of widely differing situations, e.g., endemic, family, domiciliary, neighbourhood, as a result of spare-time work, etc. An environmental tumour can, in the first place, be defined as a non-occupational tumour; on the other hand, the exposure itself is in many cases the same as under occupational conditions, and although it may be less intensive, it is sometimes even longer lasting. Thus one can consider environmental exposure to be simply a slighter form of occupational exposure, but with ill-defined borderlines (Langer & Selikoff, 1971).

(2) Since the cause of cancer development is still unknown, and since a great many individual factors in our environment are considered to be conjunctly responsible for the complex cancerisation process, it is difficult to single out one group of possible agents, namely the fibriform silicates.

While environmental asbestosis of the lungs was found to exist in animals as early as 1930 (Stewart, 1930; Schuster, 1931), pleural plaques and other pleural manifestations due to asbestos dust inhalation in man have been recorded in the literature only during the past 30 years (Meurman, 1966). There are some regions in which there is a high number of plaque-bearing subjects whose asbestos dust exposure is not of an occupational nature but is due to neighbourhood exposure, for example, in the vicinity of asbestos mines or factories, or of ship-building centres (Kiviluoto, 1960; Anspach, 1962; Rubino *et al.*, 1968; Dalquen *et al.*, 1970).

In addition, there are regions in which the soil contains weatherworn asbestos (Burilkov & Michailova, 1970; Ginzburg *et al.*, 1970) where inhabitants or workers evince in their pleura, both radiologically and autoptically, the consequences of asbestos dust inhalation. Cases have also been observed in which subjects acquired plaques through contamination from occupationally exposed members of the family (Hinz, 1968). It also seems likely that plaques may be the result of spare-time work with asbestos-containing products. Pleural plaques must hence be looked upon as important indications in the detection of environmentally jeopardised population groups (see below).

Information concerning environmentally induced cancers is not entirely reliable; yet there is a series of reports which prompts us to consider that many tumours may be asbestos-induced, despite the absence of an occupational exposure, hence that the underlying cause must be an environmental one (Oels *et al.*, 1971).

In addition to mesothelioma of the pleura and/or peritoneum the literature also mentions bronchial carcinoma (Wagner *et al.*, 1971), some of the carcinomas of the gastro-intestinal tract (Merliss, 1971) and tumours of the haemopoietic system as asbestos-induced malignant diseases; however, so far, studies published in connection with environmental hazards have nearly always been limited to a consideration of mesotheliomas as sequels of asbestos dust inhalation (Lieben & Pistawka, 1967).

Since the ubiquitous bronchial carcinoma is more frequently seen than is mesothelioma, evidence of its asbestos-induced nature in individual cases would be considerably more difficult to obtain. These carcinomas are frequently encountered concomitantly

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with asbestosis of the lung in asbestos workers (Demy & Adler, 1967; Newhouse & Wagner, 1969), but this is far less often the case as regards mesothelioma (Elmes & Simpson, 1971). This rare tumour is found *post mortem* in from 0.018 to 0.07% of all autopsies; however, following asbestos exposure, they are observed more frequently. Unlike the dose-response relationship for bronchial cancers, a considerably reduced lower limit for asbestos dust inhalation must be assumed for mesothelioma, the magnitude of which is still open to speculation.

Our information concerning the development of mesothelioma as a result of exposure to all kinds of asbestos dust is still so meagre that we can speak of causality only with reservations. However, the correlation between asbestos exposure and consequent mesothelioma is at times so striking that there can be no doubt as to their relationship. This opens a wide field for future investigations.

A noteworthy fact in connection with the occurrence of pleural plaques is that all regions which have a known increased incidence rate of mesothelioma have at the same time a greater incidence of pleural plaques. On the other hand, there are areas where an increased presence of plaques has been noted where so far mesotheliomas are absent (Kiviluoto, 1972 [1]) or have been found only sporadically (Cartier, 1972 [2]).

In South Africa, the frequency of environmental mesothelioma cases is highest in the neighbourhood of the crocidolite mines of the North-West Cape Province. In this connection, a remarkable fact is that similar observations could not be made in the vicinity of the crocidolite mines of the Transvaal and Australia. Some cases have occasionally been found in the vicinities of asbestos processing factories in England (Newhouse & Thompson, 1965) and Germany (Bohlig *et al.*, 1970). In the past, these factories processed several kinds of asbestos among which, doubtless, was crocidolite.

Noteworthy numbers of cases have not so far been reported in the neighbourhoods of chrysotile, anthophyllite, or amosite mines; although occupationally induced mesotheliomas were described by Selikoff (1973) in a factory processing pure amosite.

A particularly striking piece of evidence is that in the neighbourhoods of shipyards in France (Tayot *et al.*, 1966), Germany (Bohlig *et al.*, 1970; Stössel *et al.*, 1972), The Netherlands (Stumphius, 1969), the United Kingdom (Wagner *et al.*, 1971), and the USA (Tabershaw *et al.*, 1970) environmental mesotheliomas have been observed, in addition to occupationally induced ones; thus, an increased incidence of mesotheliomas within the range of large shipbuilding areas has been reported, the sole cause of which has up to now been considered to be pollution of the air by asbestos dust, the high concentrations of which have been demonstrated by Harries (1971). In this connection, it is important to note that since about 1900 it is precisely crocidolite that has been used for insulation purposes in ships, particularly in war-ships (Wagner, 1968).

The exact number of asbestos-induced tumours due to environmental hazards cannot be deduced from the literature, inasmuch as occupational and extra-occupational exposure can often not be separated with certainty on the basis of the published reports. However, we must reckon with a magnitude of several hundred cases, which is a sufficiently alarming number to put us on the alert for the future.

An important possible cause of asbestos-induced cancers may be found in connection with asbestos spraying as used for fire, heat, or sound insulation in ships, skyscrapers, apartment houses and car bodies

Table 1. Criteria for asbestos induced environmental cancers

1. Histology:	proved by: biopsy necropsy	malignant mesothelioma? bronchial carcinoma? carcinoma of the GI-tract?
2. History:	occasions for asbestos contacts: (*a*) endemic: asbestos mining? soil? (*b*) neighbourhood: factories? shipyards? asbestos spraying? (*c*) domiciliary: family members, inmates of the house } occupationally exposed? (*d*) due to spare-time work: handling asbestos-containing products? (*e*) other sources?	
3. Time:	Onset of exposure at least 15 years prior to tumour manifestations (normal latent period = 20–40 years).	
4. Frequency:	Analogous cancers following a similar exposure should be more frequent in the respective regions than in the rest of the population.	
5. Evidence of asbestos:	Asbestos in tumoral tissue can but seldom be demonstrated. Its presence in lung tissue ought to be recorded quantitatively (for instance following incineration).	

[1] Personal communication.
[2] Personal communication.

(Lumley *et al.*, 1971). While the asbestos sprayers themselves are nowadays completely protected by fresh air supplies, others working nearby or persons living in the neighbourhood may possibly be exposed to severe air pollution resulting from the sprayed asbestos dust. This possibility has recently led to the partial prohibition of asbestos spraying in some states of the USA, and in Denmark.

Since negative results are not considered as evidence in medical diagnoses, the question arises as to how an environmental tumour may be diagnosed with certainty as being induced by asbestos dust. In Table 1 an attempt is made to compile the criteria required to warrant the assumption of an environmental tumour. These data must, however, be taken merely as a basis for discussion; point 5, in particular, indicates how much precise knowledge is still lacking for the establishment of a causal relationship.

The number of questions still open can be demonstrated by a discussion of the occurrence of mesotheliomas in Hamburg. Hamburg is a harbour town with an important ship-building industry and a number of asbestos processing enterprises, the most important of which is indicated on the map in Figure 1.

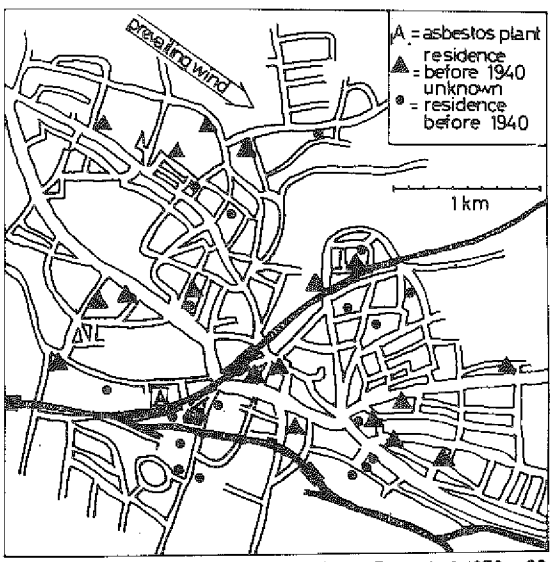

Fig. 1. Residence of 38 patients suffering from non-occupational mesothelioma due to a severe, snowfall-like asbestos air pollution in the environment of a factory which used to blow dust into the atmosphere during the years before World War II.

While the incidence rate of mesothelioma among the general population of the town of Hamburg was found in 1969 to be 0.056% for the past decade, the incidence rate in the residential area in the neighbourhood of that factory was 0.96%! The patients, who lived within the vicinity of the plant before the Second World War, reported that a severe, visible, snowfall-like air pollution was emitted from this factory, which blew dust-laden air into the atmosphere (Dalquen *et al.*, 1969).

Those cases considered to be occupational tumours, found in former asbestos workers, were not used; thus, the map shows only the 38 non-occupational mesotheliomas. Their distribution in the neighbourhood of the factory evidently seems to depend on the prevailing direction of the wind (Fig. 1). In reference to the criteria proposed in Table 1, most of the cases shown in the figure meet at any rate conditions 1–4. Latency times range from 20 to 48 years, and medium latent periods from 30 to 40 years.

Besides the cases mentioned in our preliminary report (Bohlig *et al.*, 1970) an additional 251 proved mesotheliomas were collected from the pathology departments of the large city hospitals, histories of which could be found in 150 cases. Of these tumours, those due to occupational and those to environmental causes are being mapped, and these results will be published as soon as possible.

We feel sure that should such retrospective investigations be made in other cities similar results would be found. We consider that investigations of this sort are necessary and should be made as soon as possible, since prospective studies in regard to this problem would come too late wherever successful dust control of the exhausted air will have rendered the neighbourhoods of the factories relatively free of asbestos dust pollution (Rickards & Badami, 1971).

Since, however, asbestos dust can also get into the atmosphere from asbestos-containing products such as brake-linings and friction materials as well as from asbestos cement products, it is our duty to investigate further the possibility that this indestructible material may accumulate in our environment, and the possible health hazards that this might imply.

SUMMARY

Several occurrences of cancer development following non-occupational exposure to asbestos dust are reviewed. Some discrepancies are pointed out in reports of the appearance of pleural plaques and of malignant mesothelioma. As the fibrous silicates form only a small fraction of environmental pollution, in which many other potential carcinogens are found, it has been difficult to establish that these fibres can be a direct causative agent of any specific neoplastic disease.

A proposal is made for discriminating between asbestos-induced cancers and those due to other causes. A survey of 38 mesothelioma cases from the neighbourhood of a single asbestos plant in Hamburg during the period 1958 to 1968 is given.

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Mesothelioma in relation to exposure

F. D. POOLEY [1]

It would be of great importance to determine whether there is an association between asbestos in human lung tissue and mesothelioma, and whether a specific fibre is involved. This paper presents the results of an electron-microscope examination of lung tissue from 120 cases of mesothelioma, together with similar data obtained from the examination of lung tissue from 135 cases without mesotheliomas obtained randomly at autopsy.

The mesothelioma material has come from Great Britain, Canada, Sweden and Holland, while the non-mesothelioma cases are derived from Great Britain, Sweden and Holland only. The mesothelioma material cannot be considered to be representative of the cases diagnosed in the four countries, neither can the non-mesothelioma cases be considered to be representative of the general population. They do, however, provide some indication of the relative quantity and types of fibre to be found in these two groups.

The lung blocks have all been prepared and examined in the same way. Six μm histological sections from the various cases were prepared for examination in the electron microscope by micro-incineration and extraction using the technique described by Pooley (1972). The fibre types observed in each case were recorded, and the approximate number of each type of fibre per electron-microscope specimen grid was determined.

The specimens have been grouped into six categories of exposure; to some extent they also divided themselves into these various groups on the basis of occupational exposure. The groups are as follows:

Category 1. Fibres in excess of 10,000 per grid, equivalent to a long-term asbestos exposure, the fibre numbers being similar to those observed in asbestosis cases.

Category 2. 1000–10,000 fibres per grid, equivalent to a short-term, heavy exposure to raw fibre.

Category 3. 100–1000 fibres per grid, equivalent to an indirect exposure due to handling of asbestos-fabricated materials.

Category 4. 10–100 fibres per grid, equivalent to an intermittent exposure due to handling asbestos-fabricated materials.

Category 5. 1–10 fibres per grid, equivalent to chance inhalation of asbestos fibre and the occasional use of asbestos material.

Category 6. Zero fibres found per grid.

The last two categories are those in which the general population of the industrial countries can be said to fall. Categories 3 and 4 encompass those occupations such as welding and insulating where the fibres inhaled are generally accompanied by large quantities of other, non-fibrous dust particles. In categories 1 and 2 are included those individuals who have for long periods handled raw fibre, such as asbestos miners and processing workers.

The fibre types were identified by electron diffraction as either amphibole or chrysotile. The only cases in which it was found possible to identify the specific amphibole type were those cases in categories 1 and 2—because of the large number of fibres present in these cases it was possible to obtain powder X-ray diffraction photographs from which the amphibole fibre type could be identified. The presence of asbestos bodies in the various cases was also noted.

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RESULTS

In the tables, amphibole fibre has been abbreviated to the symbol "A" and chrysotile to the symbol "C". Where the letters "A+C" or "C+A" are used, both fibre types were present but the former was the more predominant.

Table 1. Asbestos body-positive cases, as found by electron microscopy, in the mesothelioma cases; and their classification in each category of exposure

Category	Number of cases with bodies		Fibre types detected in each of the categories which were also positive for bodies[a]			
	No.	% Positive in category	A	A + C	C + A	C
1	2	100%	2	—	—	—
2	3	100%	2	1	—	—
3	9	45%	4	4	1	—
4	4	10.6%	2	2	—	—
5	0	—	—	—	—	—
6	0	—	—	—	—	—

Number containing bodies = 18
Total number of mesothelioma cases = 120
Percentage containing A = 100%
Percentage containing C = 44%

[a] See text for explanation of symbols.

Table 1 shows that only 18 of the 120 mesothelioma cases were positive for asbestos bodies by electron-microscope examination. The cases classed in categories 1 and 2 were 100% positive; the percentage positive for asbestos bodies in each category of exposure dropped concomitantly with the fibre level, until it reached zero in category 5. One point of interest derived from the investigation was that all the asbestos bodies detected were formed on amphibole fibre. In only one of the asbestos body-positive cases were there more chrysotile fibres than amphibole. Amphibole was present in all 18 specimens containing bodies, and chrysotile was also observed in 8.

Table 2 illustrates the number of mesothelioma cases occurring within each exposure category, and also the fibre types detected within each category. The 120 cases have been divided in Tables 3–6 into the various countries from which the material was obtained, in order to show the variations in exposure categories and fibre types.

Table 7 contains the results from the 135 non-mesothelioma cases, for comparison with Table 2.

Table 2. Number and classification of total mesothelioma cases occurring in each category of exposure

Category	Number of cases	Fibre types detected[a]			
		A	A + C	C + A	C
1	2	2	—	—	—
2	3	2	1	—	—
3	20	8	8	4	—
4	38	15	8	11	4
5	48	10	9	6	23
6	9	—	—	—	—
	120	37	26	21	27

Total number of cases = 120
Number containing fibre = 111
Number containing A = 84
Number containing C = 74

[a] See text for explanation of symbols.

Table 3. Number and classification of Swedish cases occurring in each category of exposure

Category	Number of cases	Fibre types detected[a]			
		A	A + C	C + A	C
1	—	—	—	—	—
2	—	—	—	—	—
3	1	—	—	1	—
4	6	4	—	1	1
5	15	3	1	3	8
6	4	—	—	—	—

Total number of cases = 26
Number containing fibre = 22
Number containing A = 13
Number containing C = 15

[a] See text for explanation of symbols.

Table 4. Number and classification of Dutch cases occurring in each category of exposure

Category	Number of cases	Fibre types detected[a]			
		A	A + C	C + A	C
1	—	—	—	—	—
2	1	—	1	—	—
3	6	—	4	2	—
4	10	—	2	5	3
5	11	—	3	1	7
6	—	—	—	—	—

Total number of cases = 28
Number containing fibre = 28
Number containing A = 18
Number containing C = 28

[a] See text for explanation of symbols.

Table 5. Number and classification of British cases occurring in each category of exposure

Category	Number of cases	Fibre types detected[a]			
		A	A + C	C + A	C
1	2	2	—	—	—
2	2	2	—	—	—
3	11	8	2	1	—
4	17	10	4	3	—
5	6	1	2	1	2
6	2	—	—	—	—

Total number of cases = 40
Number containing fibre = 38
Number containing A = 36
Number containing C = 15

[a] See text for explanation of symbols.

Table 6. Number and classification of Canadian cases occurring in each category of exposure

Category	Number of cases	Fibre types detected[a]			
		A	A + C	C + A	C
1	—	—	—	—	—
2	—	—	—	—	—
3	2	—	2	—	—
4	5	1	2	2	—
5	16	6	3	1	6
6	3	—	—	—	—

Total number of cases = 26
Number containing fibre = 23
Number containing A = 17
Number containing C = 16

[a] See text for explanation of symbols.

Table 7. Combined number of non-mesothelioma cases from Britain, Sweden and Holland showing category of exposure

Category	Number of cases	Fibre types detected[a]			
		A	A + C	C + A	C
1	—	—	—	—	—
2	—	—	—	—	—
3	—	—	—	—	—
4	7	3	—	2	2
5	58	21	1	3	33
6	70	—	—	—	—

Total number of cases = 135
Number containing fibre = 65
Number containing A = 30
Number containing C = 41

[a] See text for explanation of symbols.

It can be seen that over 50% of these cases were negative for fibres and over 90% fall in category 5 and below. Of the mesothelioma cases, on the other hand, over 92% were positive for asbestos, and over 50% were above category 5 in exposure classification.

In Table 8 the results for both mesothelioma and non-mesothelioma cases have been broken down into categories of exposure on a fibre type basis for a better comparison of the two groups of material. In the non-mesothelioma group, chrysotile is the more predominant fibre type; while amphibole fibre is more predominant in the mesothelioma group. Only 16% of the cases in the mesothelioma group above category 5 had had chrysotile exposure, while 38% of the cases which were above category 5 had had amphibole exposure. In the non-mesothelioma group the percentage of cases above category 5 with exposure to chrysotile was 3.0%, and to amphibole 2.2%.

Table 8. Breakdown of total mesothelioma and non-mesothelioma cases into categories of exposure on a fibre type basis

	Category of exposure						
Fibre type[a]	1	2	3	4	5	6	Total
Mesothelioma cases							
A	2	3	16	25	38	36	120
C	—	—	4	15	56	45	120
Non-mesothelioma cases							
A	—	—	—	3	27	105	135
C	—	—	—	4	37	94	135

[a] See text for explanation of symbols.

Tables 3–6, showing the breakdown of the mesothelioma cases into countries, illustrate to some extent the variation in industrial handling of asbestos material, the cases from Sweden, Holland and Canada being generally lower in exposure grouping than those from Great Britain. One interesting feature of the Canadian group is that there are no cases with a high exposure level to chrysotile asbestos.

CONCLUSIONS

Although the results contained in Tables 1–8 were not obtained from statistically controlled groups, they do illustrate a number of points:

(1) The lung tissue from cases diagnosed as mesothelioma does contain larger numbers of asbestos

fibres than does tissue obtained from the general population.

(2) The more predominant fibre type detected in the lung tissue of mesothelioma cases is of amphibole asbestos.

(3) Asbestos bodies, when seen in the electron microscope, are found to be associated more closely with amphibole fibre than with chrysotile.

(4) There were no cases in the mesothelioma group with heavy exposure to chrysotile asbestos. This is interesting in view of the low level of malignant mesothelioma found in workers in chrysotile mines and mills (McDonald [1]).

[1] See p. 189 of this publication.

SUMMARY

The results of electron microscope examination of lung tissue from 120 cases of mesothelioma from Great Britain, Sweden, Holland and Canada are presented briefly and compared with 135 non-mesothelioma cases. Cases have been grouped into exposure categories based on the number of fibres per electron microscope specimen prepared, on the fibre types present (i.e., amphibole or chrysotile), and on the presence or absence of asbestos bodies. Results indicate higher levels of asbestos fibre in the mesothelioma cases than in the non-mesothelioma group, while amphibole fibre is the more predominant fibre type in the former. Asbestos bodies were found to be associated with amphibole asbestos exposure and all bodies detected were formed on amphibole fibres only. Further studies of a larger number of mesothelioma cases are needed in order to establish the differences in their fibre content and in that of the general population as a whole. Also, more sophisticated instrumentation is needed in order to confirm which type of amphibole fibre is present in the mesothelioma cases.

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Discussion summary

C. E. ROSSITER [1]

CANCERS IN RELATION TO TYPE OF FIBRE, DOSE, DURATION OF EXPOSURE AND OCCUPATION

In these discussions (opened by Dr G. W. Wright and Dr E. C. Hammond) praise was expressed for the quality of the papers presented and for the substantial advancement of knowledge since 1964, even though nobody could be content that the goal of the safe use of asbestos had been achieved.

The question as to whether type of fibre plays an important role can now be answered with more assurance. Each type has been shown to be associated with an excess of bronchogenic cancer *under some circumstances*; each, except anthophyllite, has been associated with an excess of mesothelioma; data on the excess risk of tumours of the gastro-intestinal tract do not permit assessment of the role played by fibre type.

The papers presented demonstrated forcefully that the severity of cancer experience is much greater in some occupations than in others. For example the mining and milling of chrysotile is much less hazardous than some manufacturing processes and than the use of asbestos insulation materials. The exploration of the causes of these differences will provide the answers to many of the questions on how to prevent asbestos-associated cancers. Supporting evidence was also presented from Quebec that no mesothelioma had been reported from the mining areas in the last four years; from Italy, that there was a slight excess of deaths from lung cancer among 270 ex-miners from a Ballangero chrysotile mine and no cases of mesothelioma; from Great Britain, that there had been 10 cases of mesothelioma in 1600 women working in a factory assembling gas masks containing crocidolite from Western Australia, five of whom were amongst the 200 most heavily exposed women; from Czechoslovakia, where only Russian chrysotile has been used, and where 54 asbestosis cases, eight lung cancer and two mesothelioma deaths have occurred. These figures from Czechoslovakia show an excess risk of lung cancer, but the results should not yet be taken as definitive. Results from an American textile plant operating since 1896 and using all types of fibre may be contradictory in that only two mesothelioma cases have occurred.

The evidence from studies using dust exposure assessments, even when far from adequate, has shown that the excess risk of bronchogenic cancer, and most probably of mesothelioma, is related to dose and duration of exposure. Until proven otherwise, similar relations must be assumed to contribute to the severe cancer experience of insulation workers where no such attempt to quantify dose has been made.

Epidemiological evidence is urgently required on the chrysotile mining industry in the USSR, where 25,000 workers are employed and where their health has been kept under scrutiny for a number of years. The information from this stable working group should be compared to that from other mining areas.

The biologically effective dose is predominantly the quantity of fibres retained in the lungs for substantial periods of time. This is governed not only by airborne dust concentration but also, importantly, by the dimensions and shapes of the fibres. The type of fibre and the energy applied to it as it is progressively handled could markedly influence this biologically effective dose in different occupations. In setting priorities the programme of examining lung residues in detail should be expanded so that the influence of these important factors may be determined.

Other factors must also be considered in the equation governing carcinogenesis. Smoking is known

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to be particularly important in relation to lung cancer, but probably not to mesothelioma. Some of these factors govern the dose of fibre in the lungs, but substances attached to the fibre surfaces and co-existing non-asbestos carcinogens should perhaps be taken into account.

If low levels of exposure can cause slightly increased risks, then these could be far more important in terms of effect on the total community than the great increase of risk suffered by relatively few people. It was estimated that some four million men were employed in American shipyards during the Second World War, of whom nearly three million are still alive. A 10% increase in lung cancer risk for these people and for those in the construction industry might result in some 5% of all deaths from lung cancer being related to exposure to asbestos.

The American shipbuilding effort for the war started about five years after that in Britain, so there may be an increase in mesothelioma rates, parallel to that in Britain, starting shortly in the USA.

In this connection, environmental hygiene standards are practically unenforceable for occupations such as occur in the building trade. The number of inspectors needed to enforce such standards would be impossibly large. A safe assumption must be for each worker to be aware of the possible hazards of asbestos. Much more effort should be put into the minimisation of exposure by making the worker aware. This approach is being carried through in Britain where the Environmental Control Committee of the Asbestosis Research Council in consultation with the UK Factory Inspectorate has produced a number of advisory booklets, available to asbestos users and workers, on asbestos and the avoidance of hazardous conditions.

Some questions were put to individual authors:

Dr Webster suggested that the manganese content of the asbestos dust may be relevant to the mesothelioma risk. High manganese contents found in the amosite area of South Africa and in the northern parts of the crocidolite areas may inhibit the tumours through inactivating benzo(a)pyrene. He also said that the mesotheliomas seen in the amosite area were unusual in containing large amounts of bone and cartilage.

Dr Selikoff said that of the 1092 deaths in the national survey of insulation workers, there had been histology done in 378 autopsies and in 230 surgical biopsies. Of the 213 cases of lung cancer 186 had been listed on the death certificates. There was an excess of deaths from lung cancer in the first ten years of his study although amosite was not used until 1935. This implicated chrysotile as a cause of the lung cancer risk, even though there was no marked excess of total deaths until the second decade of the study.

Further evidence was presented from The Netherlands. The mesothelioma register has recorded 140 cases since 1969 out of a total population of 13 million people. The cases were mainly over 60 years old and came from the major ports, confirming the association between mesotheliomas and shipyards. There were also urban–rural differences, but some exposure to asbestos was nearly always present.

An autopsy study on the incidence of mesotheliomas in a population in the Zurich area over 10 years produced 25 cases, only one of which was associated with asbestosis, which was acquired in Belgium. These cases occurred out of 24,083 autopsies, and it was suggested that this rate could be used as a baseline. However, it was pointed out that autopsy series can be misleading; for example a 100-fold increase in tuberculosis rate was found in an autopsy series in the Federal Republic of Germany compared with the death rate in the general population.

Dr Pooley commented on the relative lack of chrysotile fibre in the mesothelioma cases. It was unlikely that the chrysotile dissolves away completely in the lung, although physical breakdown, relocation and removal could occur. Examination of tissue from persons occupationally exposed to chrysotile has shown at least as many fibres as are found in tissue from amphibole-exposed workers, suggesting that the absence of chrysotile in the mesothelioma is not an artefact.

ASBESTOS BURDEN IN LUNGS AND PLEURA, AND ITS SIGNIFICANCE

Chairman — D. Magner

Rapporteur — I. Webster

Asbestos in lung tissue

P. D. OLDHAM [1]

Two studies have been carried out under the auspices of the IARC of the proportion of certain populations in which asbestos bodies can be found in the lungs *post mortem*. One study was concerned with temporal changes in this proportion from 1936 to 1966 in one area of London. This has been fully reported (Um, 1971) and will only be summarised here. The second study was concerned with differences from place to place in the proportion with asbestos bodies *post mortem* during the period 1967–1969.

TEMPORAL CHANGES

The Central Histological Laboratory of the former London County Council Hospitals keeps complete records and material from all autopsies and biopsies carried out since the early 1930's. By courtesy of the Director, Dr P. C. Meyer, the records were searched, and 30 μm unstained sections were cut from blocks of lung tissue in 100 consecutive necropsies of patients over the age of 20, for each of the years 1936, 1946, 1956, 1966. An additional 27 patients were included from the year 1936 before it was decided to restrict the sample size to 100.

The 30 μm sections were searched for asbestos bodies in the systematic manner developed by Um (1971), in which every field of the section was covered, and the position of each body recorded on graph paper by reference to a grid placed below the slide on which the section was mounted. Table 1 gives the numbers and proportions of patients in whom asbestos bodies were found in the four years.

The population served by the LCC hospitals was, in 1936, effectively the whole of the more central parts of London. By 1966 the coverage was restricted to the more residential parts of north and north-west London. In particular, the areas in east London where asbestos factories are sited were no longer included. In consequence, the marked gradient shown in Table 1 runs counter to the possible reduction in numbers of patients who had been occupationally exposed.

Table 1. Temporal changes. Numbers of cases examined, by sex and year of death, and numbers and percentages found to have asbestos bodies in the lungs[a]

Year	No. of cases examined		No. positive		Percentage positive	
	M	F	M	F	M	F
1936	82	45	0	0		
1946	61	39	3	0	4.9	
1956	51	49	8	6	15.7	12.2
1966	55	45	10	10	18.2	22.2

[a] Um, 1971.

The gradient was fairly consistently present in all age groups, and in both sexes. In fact good agreement between observed and expected numbers at all ages and for both sexes was obtained if it was assumed that the level of exposure to asbestos, and so the chance of having detectable asbestos bodies in the lungs *post mortem*, depended on the calendar years lived through and the total quantity of asbestos in Britain in those years. Data from the Asbestos Information Committee indicated that imports of asbestos into the United Kingdom rose almost linearly year by year from about zero in 1910 to about 150,000 tons per annum in the early 1960's. Consequently, the total amount of asbestos in the country rose in a quadratic curve (Fig. 1).

The implications of this model in terms of the prevalence by age of asbestos bodies in the lungs are shown in Figure 2. It would seem useful to examine

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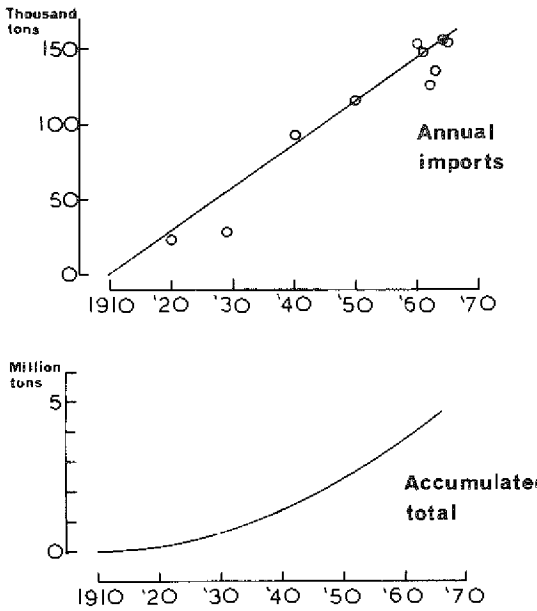

Fig. 1. Annual imports of asbestos into the United Kingdom (circles) with fitted linear trend, and consequent quadratic trend of the total accumulating there (Source: Asbestos Information Committee).

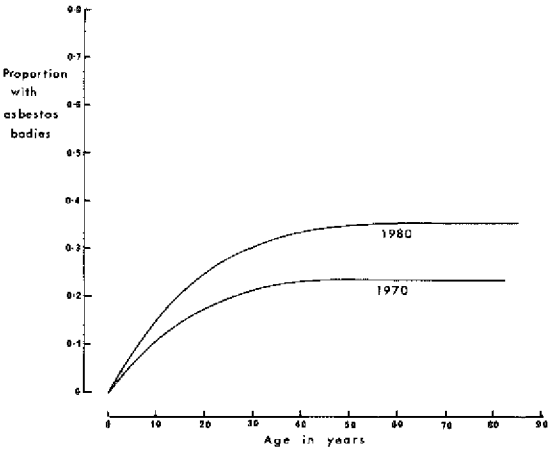

Fig. 2. Estimates of the proportion, by age, of Londoners in whose lungs asbestos bodies would be found *post mortem*.

more lungs from the 1970 post-mortem material to see if this prediction is confirmed.

Variation from place to place

A study was initiated in October 1966 to estimate the frequency with which asbestos bodies are found in the lungs *post mortem* in a variety of different geographical areas. The areas chosen depended in part on the availability of co-operative pathologists, but they were intended to cover the range from unpolluted rural areas, through urban areas of varying degrees of industrialisation, to an area of Finland in which the prevalence was known to be extremely high.

Initially, twelve centres agreed to co-operate. Two hundred consecutive post-mortems on persons over 25 years of age at each centre were to provide the material. From blocks of lung tissue taken from the base of the lower lobe, including the pleural surface, 30 μm sections were cut, de-waxed and mounted in a synthetic medium. A 1.5 cm square area of the section was to be searched by systematic traverses in alternate directions, using a 16 mm objective for scanning and a 4 mm objective for verification of the presence of asbestos bodies. The material from each centre was allocated at random among 12 observers, so that any biases of individual observers would affect the results from each centre equally.

All available material had been circulated to the observers and counted by March 1971. No centre had by then yet performed 200 post-mortems. Material was not obtained from one centre; and in another the number of cases collected and examined was too small to include. An extra observer was included to replace one who had to withdraw before finishing. It was possible to record each case only as positive or negative for asbestos bodies. The complete data, in this form, are presented in Table 2.

Three observers, b, g and k, saw rather few sections, and since each observer's potential bias has to be estimated and allowed for in arriving at comparable figures for each centre, there is a possibility that more accurate results would be got by discarding the readings of these three observers.

It is clear that for each centre only relative figures can be obtained, not absolute ones. Moreover, unless gross over-recording of asbestos bodies occurred, and if only "definite" bodies were counted, the results must still be underestimates of the true proportion of persons with asbestos bodies in their lungs, since a sample of such tissue can either contain asbestos bodies, or fail to contain them even though they occur in the whole lung.

In order to fix a base for the comparison of results

Table 2. Geographical differences. Distribution of cases studied by place of post-mortem and observer, with numbers found to have asbestos bodies in the lungs

	Centre									
Observer	A	B	D	F	G	H	K	L	M	N
a	8/20[a]	5/20	3/15	6/20	0/20	1/3	1/14	5/20	1/14	1/20
b		0/1	0/8	4/7				2/10	0/14	
c	7/20	0/19	0/7	1/7	0/22	1/10	0/18	1/9	0/6	4/17
d	10/16	1/17	1/18	8/17	0/5	1/8	7/20	5/20	4/15	2/16
e	3/19	1/12	0/14	6/18	0/15	0/3	2/14	1/21	3/20	0/20
f	3/17	2/16	1/19	5/20	0/20	0/7	1/10	2/13	2/15	0/16
g	3/12	1/9	1/13					1/3	4/12	
h	5/18	1/16	0/14	3/11	0/13	1/4	3/10	4/19	2/18	3/20
j	1/14	6/20	2/18	11/20	0/15	1/5	6/20	6/11	3/9	1/9
k	2/7	1/3	0/2	6/16			3/15	3/7	0/5	0/5
l	3/20	4/18	2/13	7/19	1/16	4/9	1/16	6/20	3/13	2/16
m	1/15	5/17	0/21	6/15	0/15	0/6	4/19	0/12	1/17	1/20
n	4/14	4/10	3/22	5/11	0/17	1/9	3/18	5/13	1/19	1/13

Totals

Observer	No. +ve/No.	%	Centre	No. +ve/No.	%
j	36/141	26	(F) Finland	68/181	38
d	39/152	26	(A) Glasgow	50/192	26
k	15/60	25	(L) London Hospital	41/178	23
l	33/158	21	(K) Dresden	31/173	18
g	10/49	20	(B) Belfast	30/178	17
a	31/166	19	(H) London RPGS	10/64	16
n	27/146	18	(M) Liverpool	24/177	14
b	6/40	15	(N) Nottingham	15/171	9
h	22/143	15	(D) Dorchester	13/184	7
m	18/167	11	(G) Galway	1/158	0.6
c	14/135	10			
f	16/153	10			
e	16/156	10			

[a] E.g., of the 20 cases from centre A (Glasgow) seen by observer a, 8 showed asbestos bodies.

Table 3. Estimates of the proportions of positive sightings of asbestos bodies, by observer and centre, with constants fitted on a probit scale for each observer and each centre

Observer	"Probit"	%	Centre	"Probit"	%
j	4.2006	21.2	(F) Finland	4.6719	37.1
d	4.1646	20.2	(A) Glasgow	4.3466	25.7
k	3.9511	14.7	(L) London Hospital	4.2390	22.3
l	4.0409	16.9	(K) Dresden	4.0355	16.7
g	4.0743	17.7	(B) Belfast	4.0053	16.0
a	3.9616	15.0	(H) London RPGS	3.9496	14.7
n	4.0317	16.6	(M) Liverpool	3.8880	13.3
b	3.6839	9.4	(N) Nottingham	3.6526	8.9
h	3.8453	12.4	(D) Dorchester	3.4703	6.3
m	3.6621	9.1	(G) Galway	2.4748	0.6
c	3.6372	8.6			
f	3.5751	7.7	Average	3.8733	13.0
e	3.5251	7.0			
Average	3.8733	13.0	Differences among centres: $\chi^2_{(9)} = 113.42$, $P < 0.001$.		

from different centres, it was decided arbitrarily to regard the data as generated by a single overall proportion positive, modified upwards or downwards by the characteristic of each centre, and upwards or downwards by the characteristic of each observer. A plausible specification for this is that there is a normal error curve representing "degree of positivity for asbestos bodies", displaced upwards or downwards by the type of material typical of each centre and by the biases of the observers, whose standard

Table 4. Estimates of the proportions of positive sightings of asbestos bodies, by observer and centre, with constants fitted on a probit scale for each observer and for five groups of centres

Observer	"Probit"	%
j	4.1256	19.1
d	4.0870	18.1
k	3.9719	15.2
l	3.9626	15.0
g	3.9468	14.6
a	3.8859	13.3
n	3.8850	13.2
b	3.7690	10.9
h	3.5907	7.9
m	3.5834	7.8
c	3.5565	7.4
f	3.4946	6.6
e	3.4450	6.0
Average	3.7926	11.4

Differences among observers: $\chi^2_{(12)} = 20.82$, $0.10 > P > 0.05$.

Group of Centres	"Probit"	%
Finland	4.6689	37.0
Glasgow London Hospital	4.2932	24.0
Dresden Belfast London RPGS Liverpool	3.9724	15.2
Dorchester Nottingham	3.5584	7.4
Galway	2.4702	0.6
Average	3.7926	11.4

Differences among groups of centres: $\chi^2_{(4)} = 113.35$, $P < 0.001$.

deviation is a function of the variability between individual cases, and whose area below a fixed point as a fraction of the whole is the proportion recorded as positive.

Table 3 shows the estimates made of these displacements, expressed as multiples of the standard deviation of the normal curve measured from an origin at +5. The corresponding percentages are also given; and these, as has been said, must be considered in relative, not absolute, terms.

In these terms they do not differ materially from the overall percentages shown in Table 2, even though the percentages for centres are at risk of distortion from the very material differences among pathologists and somewhat disproportionate numbers seen by each. It appears that the 10 centres cluster roughly into 5 groups:

Finland	38%	positive
Glasgow London Hospital	22–26%	positive
Dresden Belfast London RPGS Liverpool	13–17%	positive
Dorchester Nottingham	6–9%	positive
Galway	1%	positive

If the analysis is repeated with the centres grouped in this way, the results seen in Table 4 are obtained. The grouping of centres modifies the estimated biases of the observers, as well as giving new estimates for the grouped centres. The observers' biases become somewhat less extreme. The goodness of fit of this model is excellent ($\chi^2 = 45.59$ with 44 degrees of freedom, $0.5 > P > 0.3$). There are only three counts which differ materially from those predicted by the model, two of them by one observer.

The differences between observers, though apparently material, could in fact be no more than chance occurrences, since their significance level is about 5.3%. They should perhaps not be ignored; but if they are, the crude proportions positive can be conveniently displayed on a diagram in which the number positive appears on one axis and the number negative on the other, both on square-root scales.

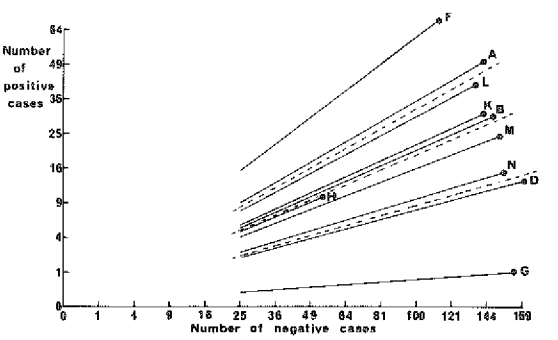

Fig. 3. The proportions of cases with asbestos bodies in their lungs in 10 different areas. Each ray corresponds to a particular percentage, and the scatter of the observed points carries the same significance irrespective of the proportion positive and the number examined.

Equal proportions then lie on rays through the origin, and the scatter of observed points is of equal significance all over the diagram. Figure 3 shows this diagram. Whereas the scatter from A to D is too large to be due to sampling variation from a single level of positivity, the three suggested central clusters are not reliably established, although the range from A to M, or from K to D, is rather too large for the clusters to coalesce in pairs. At least two distinct clusters must exist between A and D.

This clustering accords, in a general sense, with what might have been expected. A and L, Glasgow and the London Hospital, collect cases from areas which include docks and asbestos factories. K, B, H and M, Dresden, Belfast, the Royal Postgraduate Medical School (London) and Liverpool, collect cases from industrial areas which in two situations include docks, and in which there are manufacturers of asbestos products. N and D, Dorchester and Nottingham, are industrial towns without an asbestos industry. G, Galway, is of course entirely rural.

The two London centres, L and H, give proportions positive which accord well with the prediction from the results of the temporal study.

SUMMARY

Estimates have been made of the proportion of people in whom asbestos bodies can be found in the lungs *post mortem*. In the London area the proportion appears to be rising by about 1% *per annum* in persons over the age of 30. In eight areas of Britain and two on the continent the proportion ranged from 1% to 38% in 1967–69.

REFERENCE

Um, C. H. (1971) Study of the secular trends in asbestos bodies in lungs in London 1936–1966. *British Medical Journal*, **ii**, 248–251

Asbestos fibre concentration in relation to pulmonary reaction[1]

T. ASHCROFT [2] & A. G. HEPPLESTON [2]

Chemical analysis suggests that no relationship exists between pulmonary asbestos concentration and the degree of fibrosis. Counting of asbestos fibres affords another approach to this problem, and asbestos fibres in lung tissue were quantitated by macerating samples in concentrated KOH, washing the residue in distilled water, counting coated and uncoated asbestos fibres in the re-suspended sediment in a Fuchs-Rosenthal chamber, and expressing the concentration as the number of fibres per gram of dry tissue. To minimise errors several technical points were found to be important:

(1) Phase contrast microscopy must be employed to reveal fine uncoated fibres which are easily overlooked when viewed by normal illumination.

(2) Repeated washing of the macerated suspension causes loss of fine, uncoated fibres. Asbestos fibre counts in macerated suspensions after repeated centrifugation and re-suspension of the residue show that, although there was little variation in counts of coated fibres, uncoated fibres declined progressively in number with each centrifugation. We therefore chose to limit the washings to one.

(3) Drying tissue before maceration, to obtain dry weight, causes fracture of the longer coated fibres and may give spuriously high counts. It is better to macerate the tissue in the wet state; one can calculate the equivalent dry weight of tissue by drying an adjacent piece of lung showing the same pathological reaction as the test sample, and noting the change in its weight.

This technique was applied to samples of lung tissue taken from 35 individuals who were found to have asbestos bodies in histological sections, all but one of whom had a definite or probable history of occupational asbestos exposure. Thirteen cases had no histological asbestosis, 12 cases had mild fibrosis and five cases had moderate fibrosis. The series also included material from five cases of severe asbestosis, in which the extent and form of the fibrosis varied within a given lung; this permitted comparisons of fibre counts in areas which macroscopically appeared normal or showed patchy fibrosis, honeycomb change or solid fibrosis.

In the initial stages, there is on the whole a progressive rise in the fibre concentration as the severity of asbestosis increases. Mean counts of coated, uncoated and total fibres show successive and statistically significant 6–10-fold increases in the groups of cases with nil, mild and moderate asbestosis. The mean proportion of uncoated fibres also shows a small progressive rise with increasing fibrosis, but the differences between grades are not statistically significant. No relationship was found between grade of asbestosis and duration of asbestos exposure, time from first exposure to death or time from last exposure to death.

The five cases of severe asbestosis showed no such relationship to fibre concentration. Three cases (low group) had overall fibre concentrations within the range associated with mild asbestosis, and two cases (high group) showed overall concentrations within the range for moderate asbestosis. In neither group was there any correlation between the macroscopic form of the fibrosis and the fibre concentration or the proportion of uncoated fibres.

It is apparent that, while progression of disease from "no asbestosis" through "mild asbestosis" to

[1] A full account of this work appears in: Ashcroft, T. & Heppleston, A. G. (1973) The optical and electron microscopic determination of pulmonary asbestos fibre concentration and its relation to the human pathological reaction. *Journal of Clinical Pathology*, **26**, 224–234.

[2] University of Newcastle-upon-Tyne, UK.

"moderate asbestosis" is associated with a progressive increase in the fibre content of the lung, further progression to "severe asbestosis" is not associated with any further increase in asbestos concentration. Indeed, there were wide variations in asbestos concentration among areas in the same lung showing no fibrosis, fibrocystic change or solid fibrosis. These considerations, coupled with the irregular distribution of the morphological changes within the individual lung, suggest that severe asbestosis follows the intervention of one or more secondary pathological processes. There was no evidence of tuberculosis in any of the cases here considered, and it is suggested that the presence of asbestos or the reaction to it predisposes to non-specific inflammatory states which may leave residual fibrosis or honeycombing.

Other workers have shown that much of the dust in human asbestotic lungs has a very small particle size, and that most fibres are less than 0.3 μm in diameter. Such fine fibres would not be visible with the light microscope. In an attempt to estimate the proportion of finer fibres, samples of lung from six cases were macerated as before, and, after dialysing out the residual KOH and ashing the residue, a drop of re-suspended sediment was allowed to dry on a Formvar-coated grid, and the diameter distribution of the asbestos fibres was determined from electron micrographs.

Less than a third, and usually less than a fifth, of the fibres were optically visible, i.e., had a diameter of 0.4 μm or over. In this respect, there was no variation among the different grades of asbestosis. However, although the number of cases examined is small, the proportion of optically visible fibres is reasonably constant between 12% and 30% of the total, so that an optical count could be held to give a fair indication of the total asbestos concentration.

SUMMARY

Asbestos fibre concentration increases in proportion to the degree of pulmonary fibrosis as far as the moderate grade. No such correlation occurs with severe asbestosis, nor with the form the fibrosis assumes, and here secondary factors may well be concerned. Electron microscopy suggests that optically visible fibres constitute a reasonably constant proportion of the total, irrespective of the pathological reaction. Light microscopy may thus afford a guide to the total asbestos concentration.

Are ferruginous bodies an indication of atmospheric pollution by asbestos?

J. M. G. DAVIS [1] & P. GROSS [2]

The discovery that there was an association between asbestos inhalation and the occurrence of mesotheliomas led to the first detailed studies on atmospheric pollution by asbestos. Since it appeared that very short exposures to asbestos in low concentrations could produce these tumours, it was suggested that the use of asbestos-containing goods, from ironing boards to brake-linings, might be causing sufficient atmospheric pollution to endanger members of an urban population. In order to test this hypothesis, groups in different parts of the world examined lung tissue from autopsy specimens taken at random; however, since uncoated asbestos dust is extremely difficult to detect in tissues by normal light microscope methods, a count of asbestos bodies was used as an index of asbestos contamination. As methods of tissue examination became more critical, an increased percentage of lungs was found to contain bodies; and reports ranged from that of Thompson *et al.* (1963) in Capetown, who found bodies in almost 30% of a series of lungs, to the findings of Gross *et al.* (1969) who reported that 97% of lungs from a Pittsburgh series contained bodies.

However, it soon became obvious that ferruginous bodies could only be used as an index of the degree of atmospheric pollution by asbestos in a given community providing the following criteria were met, i.e., that:

(1) ferruginous bodies are specific for asbestos;

(2) a quantitative relation exists between ferruginous bodies and asbestos fibres; and

(3) the ferruginous bodies found in a lung are nucleated only by asbestos fibres inhaled with air from the community in question and not with air from other sources or locations.

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[2] Department of Pathology, Medical University of South Carolina, Charleston, South Carolina, USA.

THE SPECIFICITY OF FERRUGINOUS BODIES

Ferruginous bodies are composed of iron-containing protein and apatite deposited upon and around inhaled or injected particles of microscopic dimensions. The common feature as seen optically is the colour of the ferruginous bodies, ranging from pale straw to deep gold to red brown, contributed by their iron content; and they therefore stain deep blue for iron with acidified potassium ferrocyanide. Depending upon the shape of the particle which forms its nucleus, these bodies may be elongated, rounded, ovoid or irregular. The nucleating particles may consist of fibres, such as those of asbestos, glass or mineral wool, or of nonfibrous material (Gross *et al.*, 1968; Davis *et al.*, 1970); and these particles may be optically transparent or opaque. It is, therefore, obvious that, from a theoretical point of view, ferruginous bodies are *not* specific indicators of asbestos.

Whether, from a practical point of view, a sufficiently large percentage of ferruginous bodies found in the lungs of persons not occupationally exposed to asbestos are nucleated by asbestos fibres to allow one to ignore those ferruginous bodies nucleated by fibres other than those of asbestos has not been determined. Some studies on this problem have been published, but the results are conflicting. Thus Gross *et al.* (1969) examined ferruginous bodies from 28 cases and concluded that none contained chrysotile asbestos. However, Langer *et al.* (1970) reported that chrysotile was present in most of the ferruginous bodies found in a series of cases they examined from

New York City. More attempts to establish the asbestos load in the lungs of urban dwellers were made by Pooley *et al.* (1970) and Langer *et al.* (1971): the latter reported that chrysotile was present in 24 out of 28 cases from New York City, and Pooley and his colleagues found chrysotile in 70% of a series of cases in London. These studies were, however, not quantitative and could not clarify the relationship between uncoated fibres and ferruginous bodies.

A QUANTITATIVE RELATION BETWEEN FERRUGINOUS BODIES AND ASBESTOS IN THE LUNGS

In order to evaluate this criterion, meaningful quantitative methods based on adequate sampling of dry lung tissue by weight (10 g or more) must be employed. Methods based on the examination of only one or even of several blocks of lung tissue are inherently inaccurate and may be misleading, since the state of distension of the lung tissue is variable, and because the sampling is inadequate in terms of the total lung volume.

Method

By digesting a known weight of dry lung tissue with sodium hypochlorite, all ferruginous bodies and mineral fibres are set free. These are washed and suspended in specific volumes of water, usually so that 1 ml of suspension is equivalent to 1 g of dry lung tissue. Measured amounts are placed on cover slips, dried, and then fixed; and counts are made of the ferruginous bodies and fibres. Simple calculations convert the counts into numbers of ferruginous bodies and fibres per gram of dry lung.

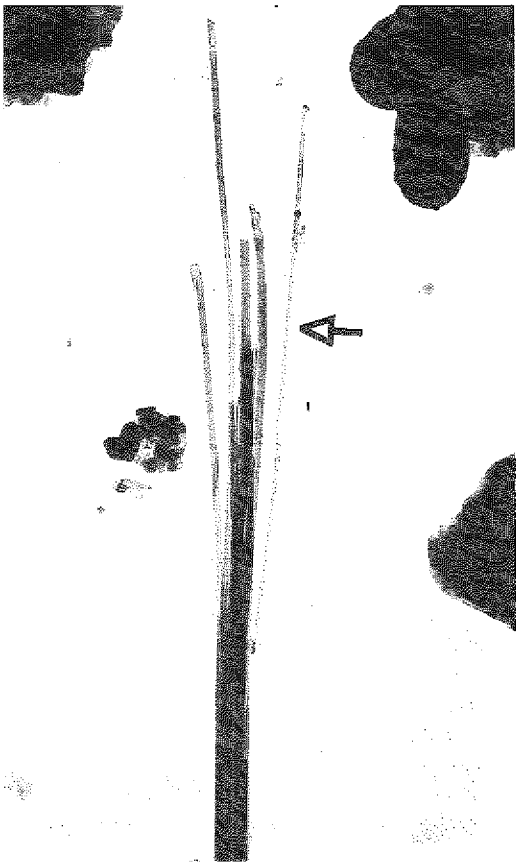
Recently, this method has been adapted for enumerating submicronic fibres. The adaptation consists of adding a known number of latex spheres to a known volume of standardised lung-sediment suspension. A droplet of this mixture is allowed to dry on an electron microscope grid. All fibres and fibrils, as well as latex spheres, are counted on each grid, and these data are similarly converted into fibres per gram of dry lung.

Using this electron microscope technique, fibres have been grouped into three categories: those fibres over 5 μm in length; those fibres less than 5 μm in length; and those fibres which, from their general

Table 1. Dust and ferruginous body counts from people not industrially exposed to asbestos

Sex	Age	Total dust[a]	Fibres[b] visible with light microscope	Ferruginous bodies[b]	Fibres visible in electron microscope[c]			
					over 5 μm	below 5 μm	Similar to chrysotile	
F	71	8.5	6,000	153	1940	4810	800	PITTSBURG
F	79	—	53,000	90	575	683	70	
F	78	6.6	34,000	309	154	875	35	
F	45	2.6	8,000	258	220	635	65	
F	60	6.5	20,000	53	630	755	60	
M	77	3.0	49,000	60	97	373	27	
M	89	33	170,000	392	318	778	187	
M	59	16	87,000	111	235	963	39	
Average		10.9	53,400	178	522	1234	160	
M	62	0.5	2,700	1,020	220	550	55	CHARLESTON
F	25	4.2	48,800	31,500	1950	4650	1100	
F	43	0.6	2,800	103	140	700	55	
M	61	7.9	4,400	51	120	380	40	
F	40	0.8	700	59	716	1083	74	
M	55	0.8	900	27	157	555	23	
F	70	3.0	1,800	188	140	306	21	
F	79	1.7	800	357	165	535	47	
M	70	0.2	900	400	58	311	6	
M	62	0.4	800	160	890	1671	104	
F	48	9.3	400	140	47	106	7	
F	39	0.5	500	—	57	110	9	
Average		2.6	5,600	3,090 (250)	389	913	128	

[a] Total dust is expressed as milligrams of dust per gram of dried lung tissue.
[b] Ferruginous bodies and fibres visible with the microscope are expressed as the numbers found per gram of dried lung tissue.
[c] Fibres visible only with the electron microscope are expressed as fibres per milligram of dried lung tissue.



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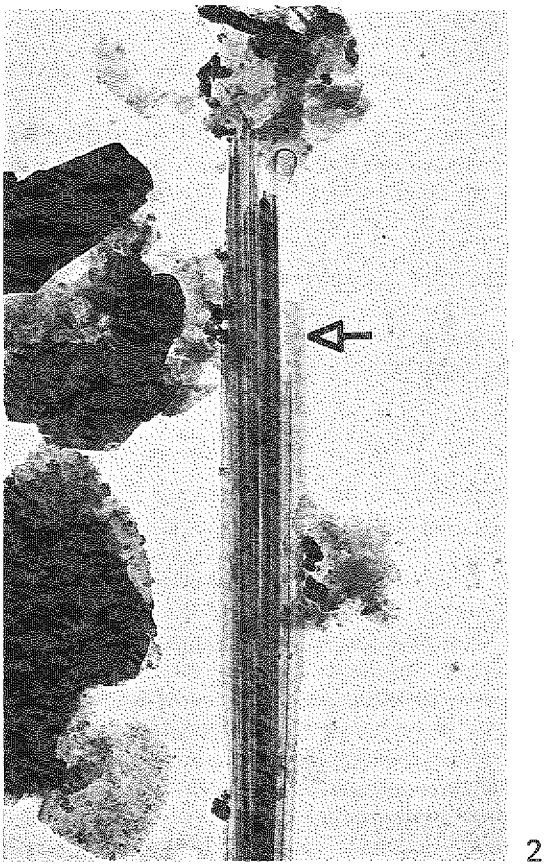

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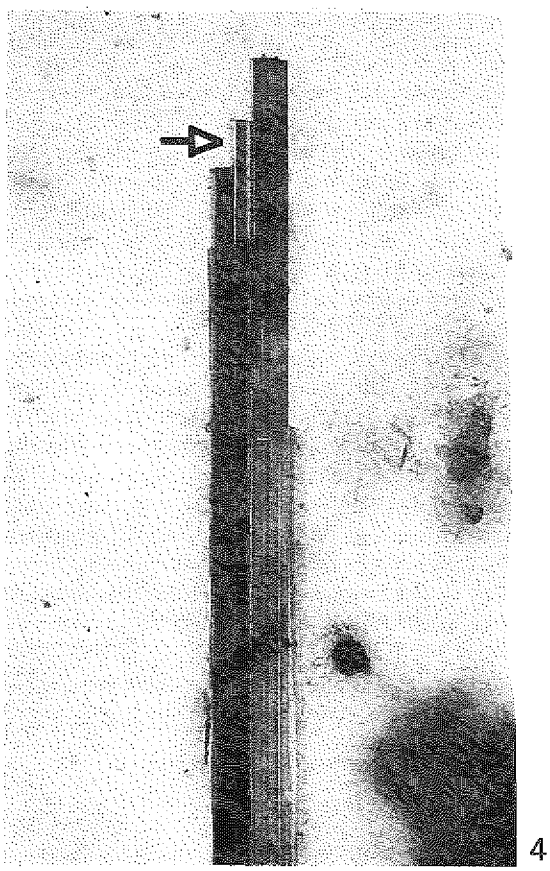

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appearance, look like chrysotile (Figs. 1–4). The structure of chrysotile as seen in the electron microscope is characteristic; but such recognition cannot be considered completely diagnostic, and specific characterisation of the chrysotile fibres is therefore necessary. This is possible with electron diffraction techniques, but the process is too time-consuming to be used on all fibres that look like chrysotile. Several hundred electron diffraction patterns are, however, being collected from a random series of such fibres, and the analysis of these patterns should give an indication of the percentage of the counts that represent true chrysotile.

Results

Studies were undertaken on the lungs of members of a normal urban population in both Pittsburgh, Pennsylvania and Charleston, South Carolina who had had no known industrial exposure to asbestos. Although the number of lungs on which optical counts, as well as electron microscope counts, have been completed is not large, the range of values obtained for both optical and submicronic fibres, as well as for ferruginous bodies, is extremely wide. In light microscope studies it has been found that the total number of mineral fibres in the lungs examined ranged between 15,000 and 400,000 per gram of dried lung. It has also been found that the fibre content of the lungs bears no constant relationship to the total lung dust content; some specimens with a very high total dust content contain relatively few fibres. The number of ferruginous bodies present in the lungs has been found to range between 28 and 4000 per gram of dried lung tissue, and the ratio of ferruginous bodies to uncoated fibres has ranged between 5: and 11,000:1.

In electron microscope examination of the dust samples the following figures have been obtained (Table 1): the number of fibres more than 5 μm long ranged between 47 and 1950 per milligram of dried lung; the number of fibres under 5 μm in length ranged between 106 and 4810 per milligram of dried lung; while the number that looked like chrysotile ranged between 7 and 1100 per milligram of dried lung. Fibres similar to chrysotile were therefore found in all the cases examined, but they made up only a small percentage of the total mineral fibre. The highest percentage found was 17, but the average was only 9%.

One can, therefore, draw the preliminary conclusion that there is no quantitative relation between the number of ferruginous bodies and the number of fibres, whether optically visible, submicronic, or those similar to chrysotile. Occasionally, we have examined lungs of people living in seacoast communities relatively free of industrial pollution in which there were high fibre counts but in which few ferruginous bodies have been found.

FERRUGINOUS BODIES IN A LUNG NUCLEATED BY ASBESTOS FIBRES CARRIED BY THE COMMUNITY IN QUESTION

This criterion could perhaps be satisfied in isolated, small, stable communities in which the inhabitants are immobile. Since such communities are few in industrialised nations, there would be very few regions in which the ferruginous bodies in the lungs of the residents could serve as an index of atmospheric pollution by asbestos, even if the other two criteria could be met.

The answer

Thus, the answer to the question as given in the title of this paper is that ferruginous bodies do not qualify as an index of atmospheric pollution by asbestos, for the following reasons:

(1) Ferruginous bodies are not specific for asbestos.

(2) There is no quantitative relation between the number of ferruginous bodies and the number of asbestos fibres in a lung.

(3) The mobility of people in industrialised communities precludes the likelihood that ferruginous bodies found in a lung are nucleated by fibres inhaled in only one community.

Figs. 1–4. Electron-microscope photographs of mineral fibres isolated from the lungs of humans not industrially exposed to asbestos. In each case the fibre consists of a bundle of crystals of a similar diameter to those of chrysotile, and the broken end of the fibre shows that the crystals break irregularly in a series of steps. In each case some of the crystals show the tubular structure that is characteristic of chrysotile (arrowed).

Magnifications: Fig. 1 × 12,500—Figs. 2–4 × 45,000

COMMENTS

Largely because of this answer, we have concentrated our attention on examination of the uncoated or naked fibres in lung tissue. One of the more significant aspects of our findings is that a sizeable percentage of the fibres found in the lungs of adults are less than 5 μm long and, on the average, about 10% of these short fibres can probably be identified as chrysotile. This, of course, does not necessarily classify the other 90% of short fibres as non-asbestos.

Short crocidolite asbestos fibres less than 5 μm long were found by Webster (1970) to be non-fibrogenic. Hilscher *et al.* (1970) came to a similar conclusion, not only with short crocidolite, but also with short chrysotile fibres. In our own studies, the preliminary results of an experiment in which 24 mg of short-fibred chrysotile was injected intratracheally showed not only that this large amount of asbestos was well tolerated by the animals, but that the tissue response was a macrophage reaction without significant stromal proliferation. This is in contrast to experience with intratracheal injections of long-fibred asbestos, where doses greater than 2 mg were regularly associated with mortality, and where a fibrosing tissue response was later demonstrable in surviving animals.

Another pertinent observation with regard to short-fibred asbestos was made several years ago during studies of ferruginous body formation (Davis, 1970). It was found that fibres which could be completely ingested by macrophages, i.e., those shorter than 5 μm, did not become coated with ferruginous material.

Thus, a degree of uncertainty exists with regard to the health hazard posed by asbestos fibres shorter than 5 μm long. This uncertainty is reinforced by a failure to associate specific pulmonary disease with the presence of asbestos dust in the lungs of people not occupationally exposed to this dust. The wide range of loads of asbestos dust encountered in such subjects, where there is no evidence of specific pulmonary disease, suggests that there is a dose-response relationship and that the level at which there is a demonstrable response has not been reached in those examined.

It is obviously necessary to determine at what level of asbestos dust in human lungs the earliest fibrogenic response can be demonstrated; this determination so far appears to be feasible only for people who have a history of *occupational* exposure to asbestos. It is even more important to determine whether the carcinogenic effect of asbestos is also dose related, and at what level of atmospheric contamination a danger of tumour formation is found. So far there are no data on this subject.

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Pleural plaques

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DESCRIPTION

Hyaline plaques of the parietal pleura should be distinguished from diffuse thickening of the parietal and visceral pleura, which may follow haemothorax and infections (especially of tuberculous origin).

Macroscopic appearance

A hyaline plaque is a yellow-white, patchy thickening of the pleura, projecting slightly above the surrounding tissue as a shiny, porcelain-like plateau. The surface may be smooth or nodular. A wide variety of shapes and sizes is seen, from small specks to almost continuous plaques covering large areas of the pleura and up to 1 cm thick (Meurman, 1966). Pleural plaques are not associated with adhesions (Meurman, 1966; Thomson, 1969) and are easily stripped from the chest wall at autopsy.

Distribution

Usually multiple, plaques are found almost exclusively on the parietal pleural surface, tending to follow the lines of the lower ribs, particularly from the seventh to the tenth (Meurman, 1966). The majority are bilateral and symmetrically arranged (Meurman, 1966), with the left side predominating (Kiviluoto, 1960). Confluence may be seen, especially in the paravertebral regions. Plaques also frequently occur on the diaphragm where they are characteristically confined to the central tendons. Occasionally, they are found on the anterior mediastinal pleura (Selikoff, 1965) and on the left cardiac border, near the point of "opposite pulsations" (Fletcher & Edge, 1970).

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Microscopic appearance

Pleural plaques are non-neoplastic and consist of collagen (Roberts & Ferrans, 1972) which is arranged so as to give a characteristic "basket-weave" appearance which ceases abruptly at the plaque margin. In the mature lesion there are few fibrocytic nuclei; they are avascular, and free from inflammatory cell infiltrate. In a developing plaque, growth appears to come from the deepest aspect where there is some vascularity, plumper fibroblast-like cells and sometimes a limited infiltrate of lymphocytes and plasma cells (Thomson, 1969).

The mesothelial cells lining the normal pleura extend over the plaque surface in continuity, thus rendering the plaque an "extra-pleural" structure (Thomson, 1969). Occasionally, fibrin-like material is seen on the mesothelial cell surface (Smith, 1972[3]).

Calcification

Meurman (1966) demonstrated the presence of calcium deposits in 87% of consecutive autopsies with pleural plaques. Dystrophic calcification may be seen in zones of degenerated collagen, usually in centrally situated areas of poor nutrition. Histologically, calcium is distributed as fine granules in irregular accumulations in different layers of the plaque.

Radiological features

In the standard postero-anterior radiograph, tangential views of plaques are obtained at the charateristic sites on the lateral chest walls, the domes of the diaphragm, and the pericardial and paravertebral pleura.

Well-calcified plaques can be seen on the anterior and posterior chest walls, the appearances varying

[3] Personal communication.

with the extent of calcification (Kiviluoto, 1960). It may be difficult to distinguish early, small, individual nodules from intrapulmonary opacities (Anton, 1967). Later, a more obvious multinodular outline is visible, becoming continuous to form the so-called "holly leaf" pattern (Hourihane *et al.*, 1966). Large plaques present more confluent, dense opacities of bizarre shapes.

Precalcified hyaline plaques are less easily seen *en face* (Anton, 1968); but with suitable techniques, they are well demonstrated tangentially, especially in line with the lower ribs on the lateral chest wall, and on the dome of the diaphram (Fletcher & Edge, 1970).

Discrimination between poorly-defined hyaline parietal pleural plaques and the more diffuse and less specific type of pleural thickening involving the visceral pleura can be difficult in some cases. Also, the smallest hyaline plaques on the lateral chest wall may be confused with the normal soft tissue companion shadows. For these reasons the calcified pleural plaque remains the more precise diagnostic marker, especially in comparative epidemiological studies using a single postero-anterior film.

The revised international classification of radiographs (ILO, 1972) meets this requirement by providing two separate indices of pleural abnormality, one for pleural calcification and the other for pleural thickening of all types. The much less specific minor fibrosis of the costo-phrenic sulcus is noted separately.

RELATION OF PLEURAL PLAQUES TO ASBESTOS EXPOSURE

Pathological aspects

Meurman (1966, 1968) has reported plaques in 40% of subjects over 15 years of age, some of whom were exposed to anthophyllite asbestos in the Finnish mining communes. Asbestos bodies were present in the lungs of 86% of subjects with bilateral plaques. Forty per cent of cases with asbestos bodies did not have plaques; but if asbestos was present in abundance, plaques were regularly seen. This observation has been confirmed by Hourihane *et al.* (1966), Roberts (1967), McPherson & Davidson (1969) and Mattson & Ringqvist (1970). Robinson (1972), however, has been unable to confirm such a correlation.

Meurman (1966) was unable to detect asbestos bodies within the plaques, but asbestos fibres have subsequently been identified (Hourihane *et al.*, 1966; Thomson, 1969).

Radiographic surveys

By comparison with autopsy series, it is inevitable that radiographic surveys will underestimate the true prevalence of pleural plaques.

(1) *Occupational exposure.* Pleural calcification has been observed in asbestos workers in Dresden (Jacob & Bohlig, 1955; Anspach, 1962), London (Hourihane *et al.*, 1966), Bulgaria (Gerasimov *et al.*, 1967) and in Czechoslovakia, where the exposure is mainly to Russian chrysotile (Navratil, 1970a). An overall prevalence of approximately 5% is reported.

In asbestos insulators with over 20 years since first exposure, pleural calcification was observed in 11 of 28 men in Copenhagen (Frost *et al.*, 1956). A prevalence of 38% was reported in a large series in New York City (Selikoff, 1965). In Belfast, where insulators spend much of their time in ship-building, the prevalence was 14% (Langlands *et al.*, 1971). In United Kingdom naval shipyards the prevalence was 5% (Harries *et al.*, 1972), but calcified plaques were also seen in 1.6% of the large number of neighbourhood workers in these yards. Similar observations have been made among New York City ship repair workers (Ferris *et al.*, 1971).

A survey of men aged 36–65 working in Quebec chrysotile mines and mills (Rossiter *et al.*, 1972) showed a prevalence of pleural calcification ranging from 0.5% in the youngest five-year age group to 9.2% in the oldest five-year age group. There was a marked disparity in the average prevalence between two groups of mines in this area (0.4% at Asbestos; 5.2% at Thetford). In a comparable study of chrysotile miners and millers in Cyprus (Constantinides, 1972 [1]) the prevalence was 5.9%.

Pleural plaques have also been observed in South African crocidolite and amosite miners (Oosthuizen *et al.*, 1964), Italian chrysotile miners (Vigliani, 1969) and Finnish and Bulgarian anthophyllite miners (Kiviluoto & Meurman, 1969; Zolov *et al.*, 1967). Earlier reports have described pleural calcification in tremolite talc miners (Siegal *et al.*, 1943), and in workers handling talc and mica (Smith, 1952).

(2) *Environmental exposure.* A high prevalence (8%) of pleural plaques has been recorded in pioneer

[1] Personal communication.

surveys of non-occupationally exposed populations living in the neighbourhood of anthophyllite mines in Finland (Kiviluoto, 1960). Plaques have also been found in the vicinity of crocidolite and amosite mines in South Africa (Sluis-Cremer & Theron, 1965) and near a chrysotile mine in Italy (Vigliani, 1969).

Calcified plaques have been found in Bulgarian tobacco farmers, who till soil containing anthophyllite (Burilkov & Babadjov, 1970). Isolated instances of plaques associated with this type of exposure have been reported from Finland (Kiviluoto, 1960).

Plaques have been reported in non-occupationally exposed persons living in the vicinity of asbestos factories in Dresden (Anspach, 1962), London (Hourihane *et al.*, 1966), Hamburg (Dalquen *et al.*, 1970) and Czechoslovakia (Navratil, 1970a). The same authors also quote cases among relatives of exposed workers.

(3) *Surveys of non-exposed groups.* In Finland a control population of 7101 was examined by Kiviluoto (1960), who reported that the only pleural calcifications found were attributable to previous tuberculous pleurisy. Wallace & Langlands (1971) compared 50 carefully matched non-exposed controls with 50 insulators and found no calcification in the controls, but 21 cases among the insulators. A comparable examination of 1896 men living near, but not working in, Devonport dockyard produced one case of unilateral grade I calcification (Harries *et al.*, 1971 [1]).

The use of hospital out-patients as controls is unsatisfactory because of the frequent occurrence of pleural calcification arising from other causes (Kendall & Caplin, 1967). The difficulty becomes greater when evidence of non-calcified pleural thickening is also investigated (Kiviluoto, 1969); this can be seen in the inconclusive results of the co-operative study of British chest clinic cases of pleural thickening quoted by Gilson (1969).

A relationship to asbestos exposure has not been established in a small proportion of cases found in mass radiography surveys (Anspach, 1962; Neef, 1963); and in Czechoslovakia a high local prevalence of pleural calcification has been observed in the older members of small groups of farming families (Hromek, 1962; Marsova, 1964).

Relation to dust exposure

In the Quebec and Cyprus mining surveys (Rossiter *et al.*, 1972), careful measurement of total dust exposure and more sophisticated statistical analysis led the authors to conclude that, given a certain minimum initial dust exposure, pleural calcification is related only to age.

Interval between first exposure and plaque formation

The youngest recorded case of pleural plaque formation was 18 years old (Meurman, 1966), the subject having been environmentally exposed in an anthophyllite mining area. Radiological evidence of these plaques is rare in subjects under the age of 30 (Kiviluoto, 1969). An interval of 20 years from the onset of occupational exposure is usually necessary before recognisable calcification occurs (Selikoff, 1965). Two Belfast insulators under 30 years of age were found to have plaques, but both had been exposed to asbestos in childhood (Langlands *et al.*, 1971.)

CLINICAL EFFECTS

Pleural plaques, in the absence of other abnormalities, give rise neither to symptoms nor to abnormal physical signs and are not accompanied by defects of lung function (Leathart, 1968).

They are commonly seen concurrently with pulmonary fibrosis, diffuse pleural fibrosis and mesothelioma, but they do not influence the clinical course of these conditions. Effusions have been observed (Sheers & Templeton, 1968), but these are probably due to a coexisting pleural reaction of the diffuse type. A higher than expected incidence of bronchogenic carcinoma when plaques are present has been reported by Fletcher (1972).

PATHOGENESIS OF PLEURAL PLAQUES

Kiviluoto (1960) suggested that plaques result from an inflammatory response of the parietal pleura to mechanical irritation by fibres projecting through the visceral pleura of the lung. The drawback to this explanation is the absence of surface inflammatory reaction, and also a total absence of adhesions.

Enticknap & Smither (1964) and Hourihane *et al.* (1966) have postulated the intracellular carriage of asbestos particles within lymphatic vessels to the parietal pleura. Certainly the sites of plaque formation coincide with pathways of lymphatic drainage

[1] Unpublished data.

(Lemon & Higgins, 1932), and the selected sites are possibly zones of relative stasis. The massaging effect of the diaphragmatic muscle and intercostal muscles may result in dust particles being "washed up on the shore" of the inactive central tendon and of the pleura overlying the lower ribs, respectively. Experimentally, Roe *et al.* (1967) showed that subcutaneous injections of asbestos caused apparently specific transport of fibres to the submesothelial cells of the thorax and abdomen; and Godwin & Jagatic (1970) have confirmed that fibres become widely distributed throughout the body.

Thomson (1969) noted that the mesothelial cells play no part in plaque formation, but that development appears to come from its deepest aspect. He suggested that the distribution of plaques results from the downward and outward gravitational movements of the sharp asbestos fibres. The relatively extensive lesion developing from a very small number of fibres suggests a sensitivity reaction.

In support of an immunological basis for plaque formation, Pernis *et al.* (1965) described an increased prevalence of rheumatoid factor in asbestos workers. Turner Warwick & Parkes (1970) reported a fourfold increase in the incidence of antinuclear factor and rheumatoid factor in those patients with asbestos exposure referred to the London Pneumoconiosis Medical Panel, over the incidence in random populations. Levels of serum gamma globulins are higher in asbestos-exposed persons with plaques than in those without, and higher still than the level in non-exposed controls (Navratil, 1970b).

CONCLUSIONS

(1) There is ample evidence of an association between pleural plaques and all types of exposure to asbestos, and to all types of asbestos fibre. Asbestos is not the only cause of plaques but it is certainly the most common.

(2) There is insufficient evidence to establish a direct cause-and-effect relationship, and intermediate or additional factors may be involved.

(3) The prevalence of plaques is not related to total dust exposure; but, given a minimum initial exposure, the prevalence depends on age.

(4) Pleural plaques are not harmful clinically, but they act as a useful marker to draw attention to possible asbestos exposure.

(5) The pathogenesis of plaque formation is unknown.

FUTURE RESEARCH

(1) A better understanding of the mode of transport and distribution of asbestos fibres within the body is required. Electron microscope identification of fibres in pleural fluids and the charting of their distribution within the structures of the chest wall, particularly in relation to soft, mobile structures and firm, immobile areas may clarify the mode of plaque distribution.

(2) Identification of the different types of asbestos fibres should be made from autopsy material from patients with pleural plaques.

(3) Serial immunological screening of exposed groups should be carried out to determine if changing immunological reactions are related to the development of pleural plaques or other asbestos diseases.

(4) The factors concerned in the discrepancy of pleural plaque prevalence in the two groups of mines in Quebec require elucidation.

(5) Monitoring, in selected areas, of the increasing number of subjects who have been exposed over the previous 30 years and who are now entering the appropriate age zone should be continued in order to assess the incidence of pleural plaque formation and other biological effects of asbestos exposure.

SUMMARY

Hyaline plaques of the parietal pleura, with their characteristic distribution, should be distinguished from diffuse fibrous thickening of the parietal and visceral pleura.

A strong correlation exists between plaque formation and exposure to asbestos dust of all types, but an immediate cause-and-effect relationship has not been established. A latent interval of 20 years between first exposure

and evidence of plaque formation is usual; but the prevalence of plaques is more dependent on the age of the patient than on the total dust exposure. Those environmentally exposed from birth develop plaques at an earlier age than those occupationally exposed. Despite radiological advances in the identification of hyaline plaques, calcification remains the most reliable marker for group studies.

The pathogenesis of pleural plaques is unknown, but investigation of the transportation of asbestos fibres to selected sites within the body, and immunological studies, would seem to be useful projects for the future.

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Structure and composition of pleural plaques

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The study described below is of human pleural plaques taken from patients occupationally exposed to asbestos dusts; for purposes of comparison plaques taken from patients with calcifications of completely different etiology, such as tuberculosis or emphysema, were studied.

MACROSCOPIC APPEARANCE

The specimens studied were taken preferably from the parietal pleura and had the well-known macroscopic appearance of pearly-white plaques, firm to the touch, a few millimetres thick with an area ranging from a few square centimetres to about 50 cm^2. In most cases the free side of the plaque had a mammillated surface.

Seen in section both the plaque and the mammillae have a laminated texture which can clearly be seen under the magnifying glass. Examination in section also reveals possible calcifications. These almost always appear in the median plane of the plaque and in the centre of the mammillae. If the calcification is already compact, it can be clearly differentiated from the neighbouring parts of the plaque and can be cut away from them. The calcified zones may not be clearly visible in fresh tissue, but if they are kept in formalin for some time they can be distinguished by a markedly yellow colour.

HISTOLOGY

Pleural plaques are made up of fibrohyaline connective tissue, practically free of cells, made up essentially of thick bundles of collagen fibres separated by optically empty pseudolacunae. They are bounded at their lower edge by a thin network of elastic fibres which is stained by orcein and which itself lies on subjacent adipose tissue. The surface, which is bounded by endothelium, is frequently covered with nodules in which collagen fibres are arranged concentrically, like onion rings.

There are only a few cellular elements, mainly fibroblasts, inside the plaque itself. Inflammatory type cells are never encountered. On the other hand, in the peripheral zone at the edge of the plaque, small agglomerations of lymphoplasmocytes or histiocytes or of mast cells can be seen in the vicinity of small vessels.

The calcifications are usually situated in the collagenous zone; and in section, after staining with von Kossa stain or following micro-incineration, they show up as flattened nodules or thin laminae of varying area, or as more diffuse deposits. They are preponderant in the central or deeper zone of the plaque, and they are separated from the endothelium by a relatively thick layer of non-calcified fibrohyaline tissue.

ULTRASTRUCTURE

The fibrous and calcified zones of the plaques will be considered separately.

Fibrous zone

Whatever the size of this zone in relation to the pleural plaque as a whole, a certain number of common ultrastructural characteristics are found in all the cases examined, with only a few variations.

Electron microscopy first of all confirms the structures that can be seen under the light microscope, i.e., bundles of collagen fibres embedded in a hyaline matrix (Fig. 1). These fibres usually show the normal periodicity of collagen (64 nm); on the other hand, some of them have a markedly larger diameter

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than the general average (200 nm as compared to 30–70 nm) (Fig. 2). Also, some cell debris is encountered embedded between the collagen layers.

In addition to these organic components, numerous electron-dense particles can be seen scattered in the fibrohyaline tissue (Figs. 3 & 4). Some of these particles are solid or laminated rounded bodies with a diameter between 0.1 and 0.5 μm. Others, also compact and of similar dimensions, have a marked fibrous external structure which consists of very fine needles with diameters of the order of 7.5 nm. Finally, other types of markedly differentiated dense particles are found, some of them in the form of spheroid or ovoid plaques of fragmented lamellated appearance with a diameter of the order of 1 μm, and others as bundles of acicular crystals of very varied dimensions.

These different types of electron-dense bodies represent incipient calcifications. They are soluble in acids, and most of them produce an electron diffraction pattern corresponding to that of whitlockite in the case of the compact or lamellar ovoid forms and of apatite in the case of acicular crystals.

Examination under high magnification after lead staining shows dark granular patches with incipient crystallisations or impregnating crystalline masses in the course of growth.

It should be noted finally that the nascent calcifications, particularly those of the whitlockite type, are situated mostly in zones containing cell debris. However, early calcification of the apatite type can also be seen along the length of the collagen fibres.

An important point to note is the similarity between these structures and those encountered in the same subjects in the thickened visceral pleura, where fibrohyaline tissue enclosing cell debris and rounded electron-dense bodies made up essentially of whitlockite can also be observed.

Calcified zone

This zone is characterised by an invasion of the fibrohyaline tissue by acicular apatite crystals, among which whitlockite nodules persist (Fig. 5). The crystals apparently grow along the length of the collagen fibres whose underlying periodicity is still visible in many places.

Phosphotungstic acid or hydrochloric acid dissolve the apatite and allow the collagen fibres to be seen (Fig. 6).

Lacunae may persist among the agglomerations of apatite crystals; in these a fairly loose-woven fibrohyaline tissue can be seen which is found with lead staining to consist of a fine reticulation. These lacunae also contain isolated apatite crystals.

Comparison of pleural plaques and pulmonary calcifications of different etiologies

As was stated above, there is a marked similarity between the calcifications found in the fibrous zone of a parietal pleural plaque and those seen in the thickened visceral pleura of the same individual.

To determine whether this similarity applied to other types of calcified lung tissue, we examined three specimens from pathological calcifications of totally different origins, i.e.,

(i) a fibrohyaline plaque from a subject whose occupation apparently involved no exposure to asbestos dust (a search for asbestos fibres, made on the basis of the method described later, proved negative);
(ii) a gas cyst; and
(iii) a tuberculous caseification.

In all three cases electron-microscopy revealed the presence of electron-dense rounded bodies against a background of cell debris and of collagen or elastic fibres, according to the type of lesion. The spheroid calcifications sometimes had a laminated appearance. When oriented crystals were present, these needles grew among the structured elements of the fibrous weft, the collagen or the elastic fibres.

Altogether these calcifications showed a marked similarity to those of pleural plaques.

COMPOSITION OF THE PLEURAL PLAQUES

Fragments of pleural plaques were analysed for their contents of collagen, phosphate, calcium, sulphur and hexosamines. Measurements were made separately in the fibrous zone and in the calcified zone.

Collagen

Specimens were first decalcified, and the collagen level was determined after acid hydrolysis by measurement of hydroxyproline. Table 1 shows the collagen values in the decalcified tissue and, for purposes of comparison, the concentrations in a fibrohyaline plaque from a subject not exposed to asbestos dust.

There appears to be no significant difference between the collagen levels in the two zones of the calcified pleural plaques. Moreover, the proportion

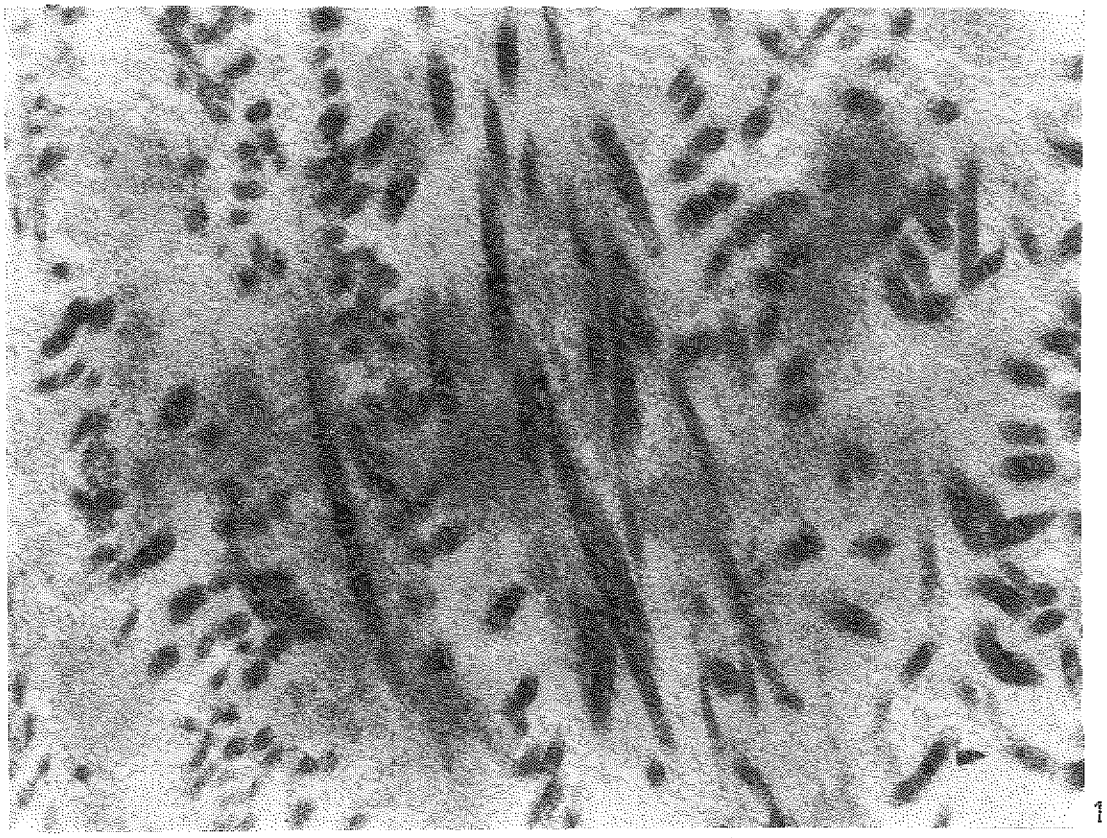

Fig. 1. Parietal pleural plaque—fibrous zone—collagen fibres in the fibrohyaline tissue.

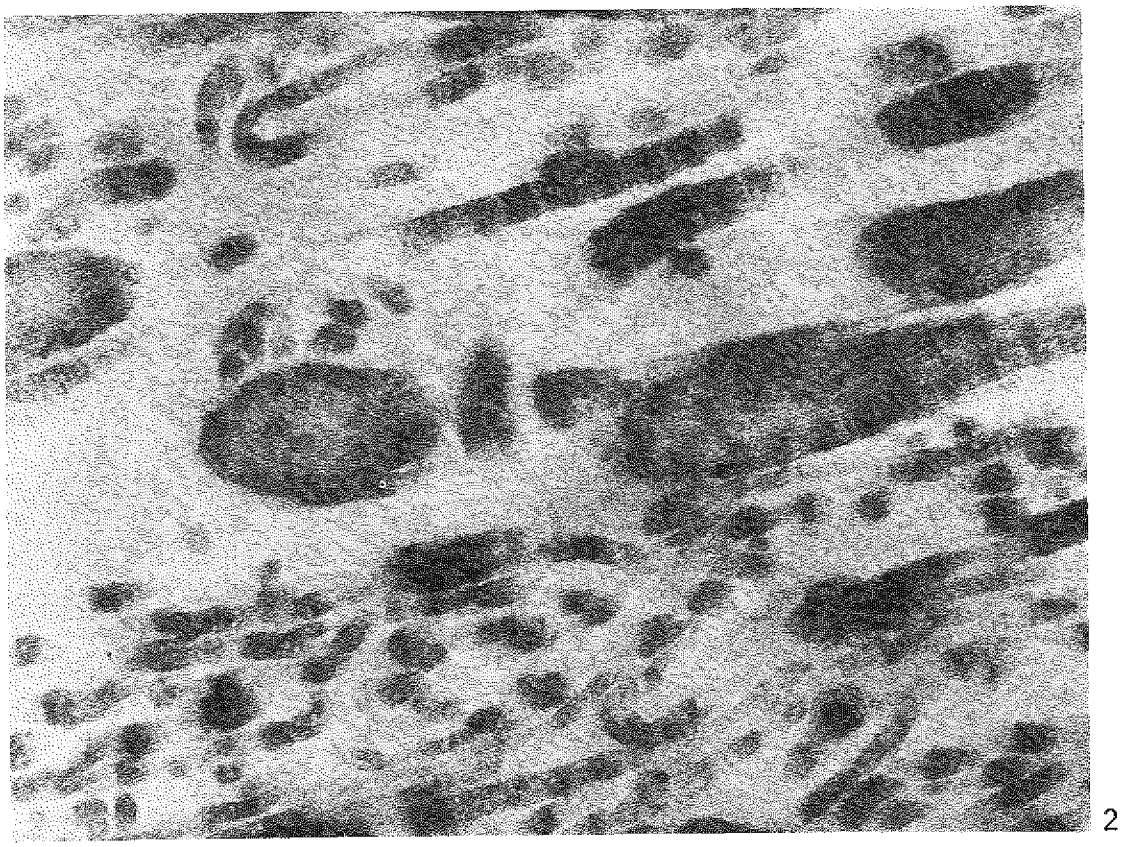

Fig. 2. Parietal pleural plaque—fibrous zone—collagen fibres with large diameter.

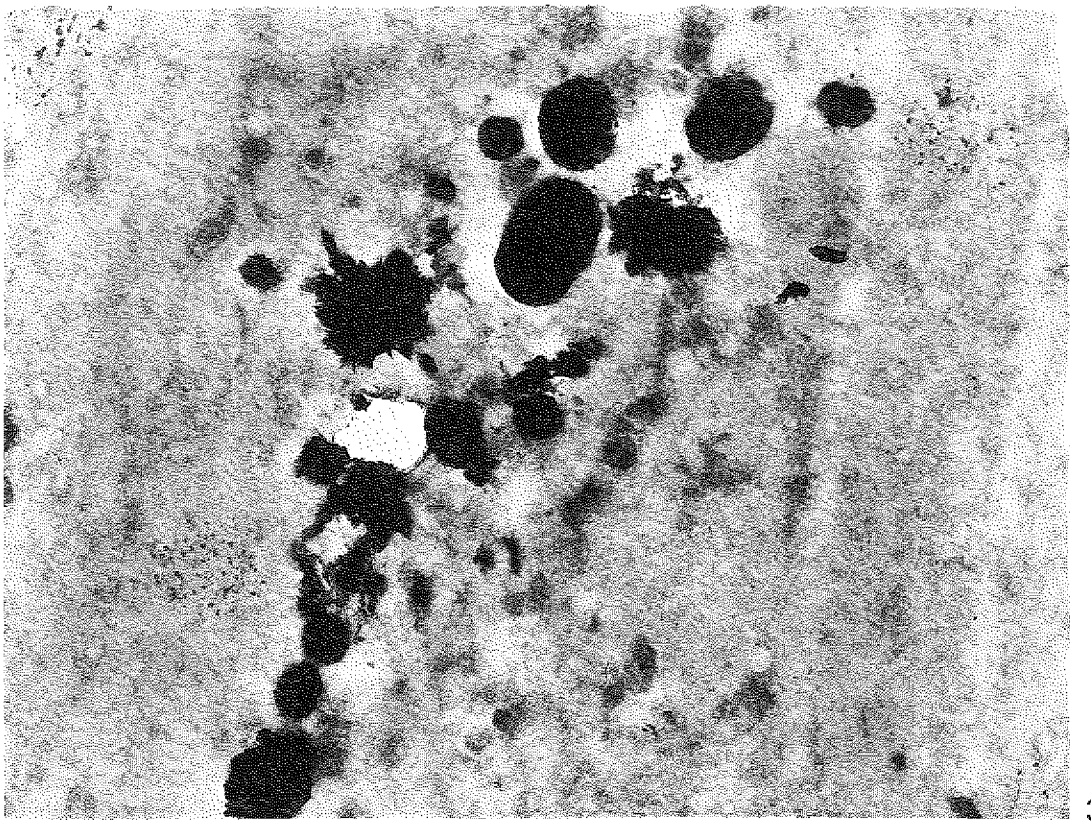

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Fig. 3. Parietal pleural plaque—fibrous zone—incipient calcifications—A → whitlockite; B → apatite; C collagen

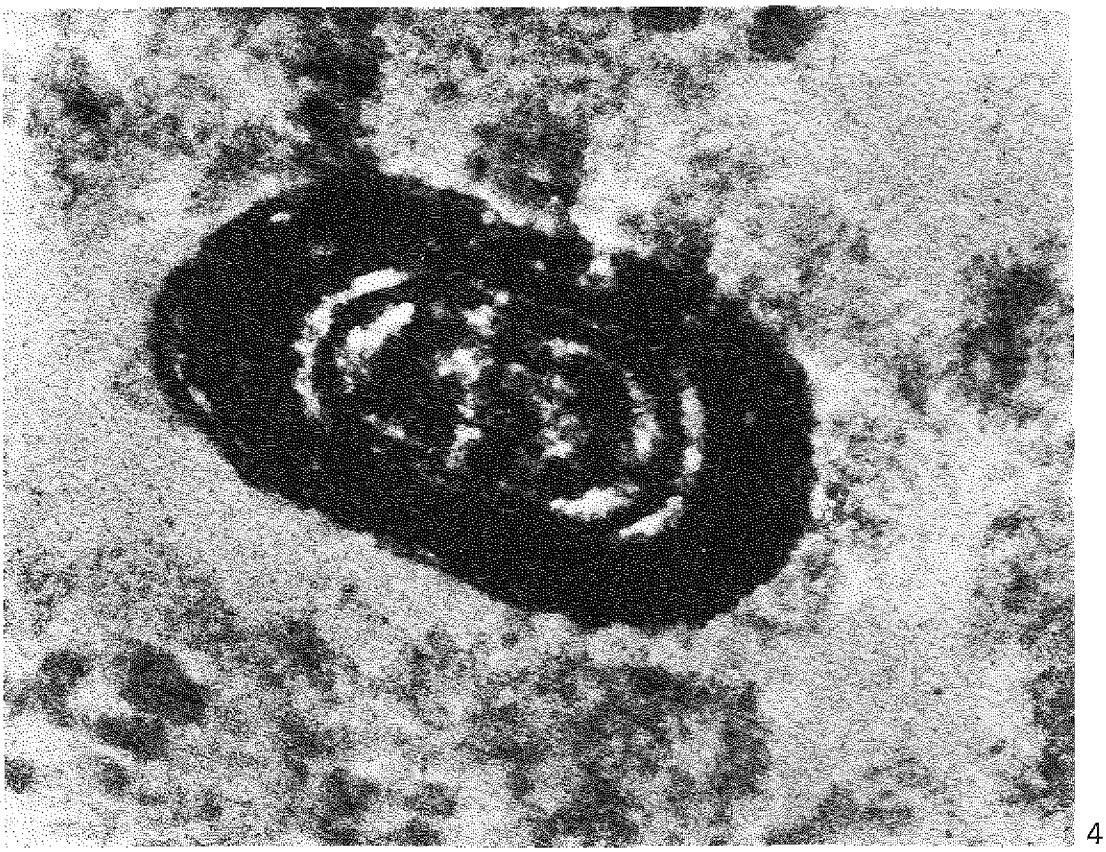

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Fig. 4. Parietal pleural plaque—fibrous zone—lamellated structures.

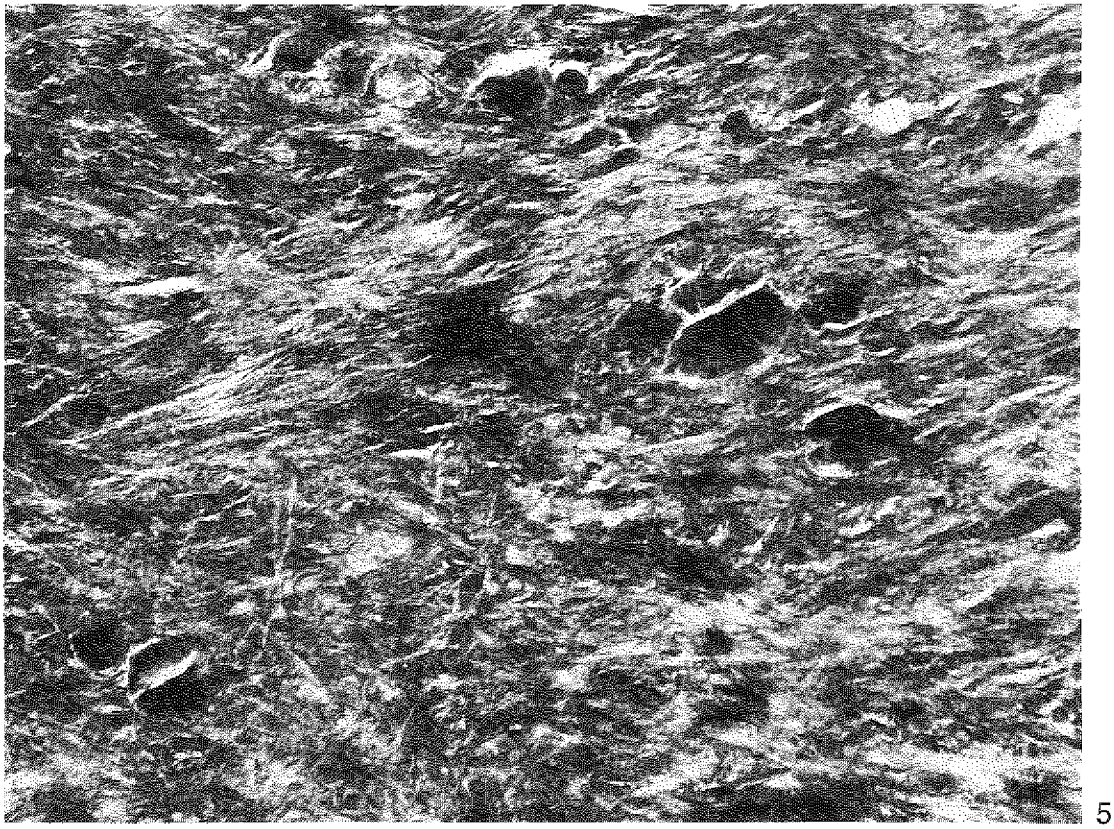

Fig. 5. Parietal pleural plaque—calcified zone—massive calcifications—A→whitlockite; B→apatite; C→periodicity of collagen.

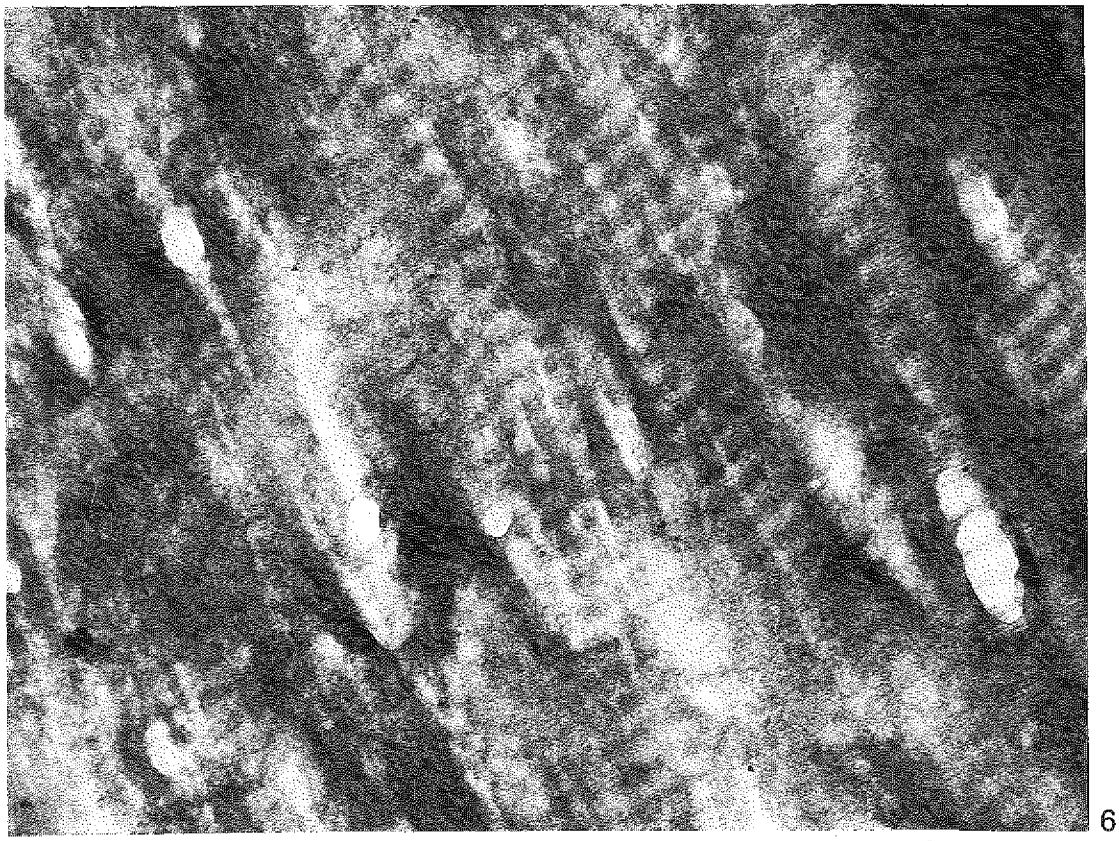

Fig. 6. Parietal pleural plaque—calcified zone—collagen fibres after decalcification by phosphotungstic acid.

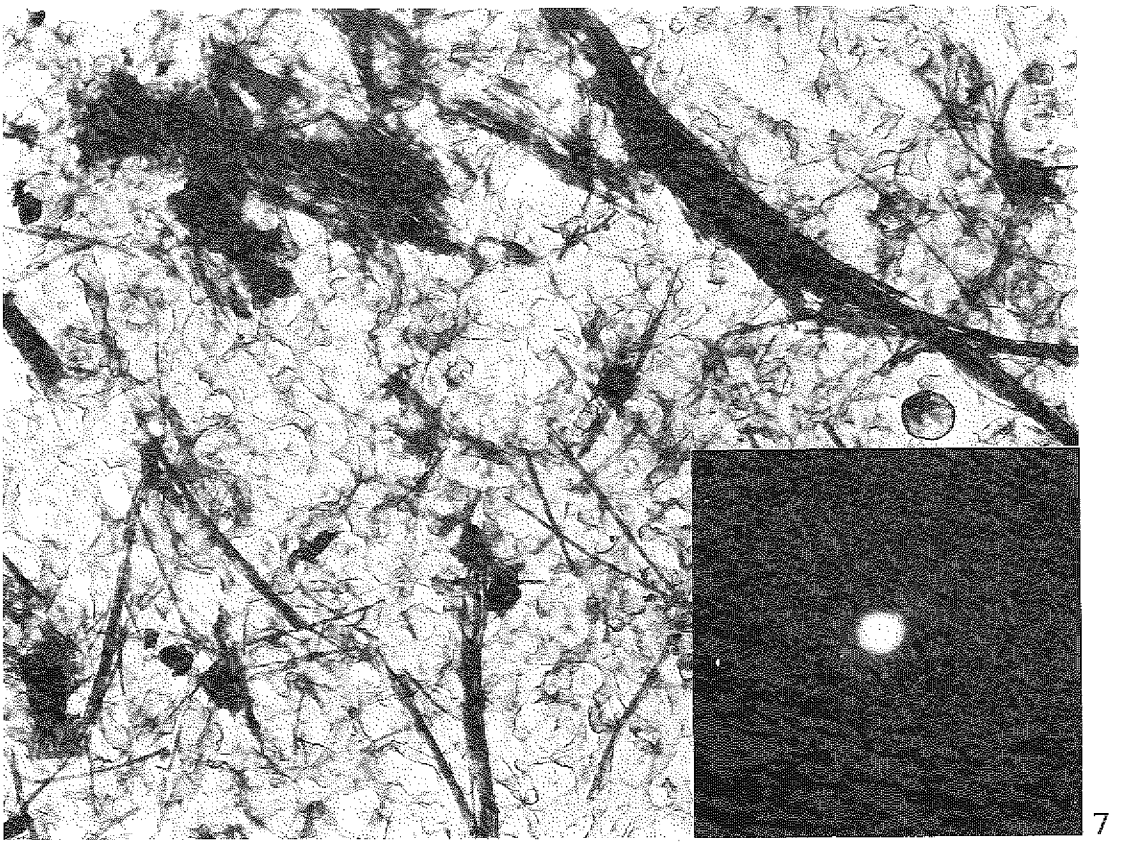

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Fig. 7. Parietal pleural plaque—calcified zone—chrysotile fibres visible after incineration and washing with acid.

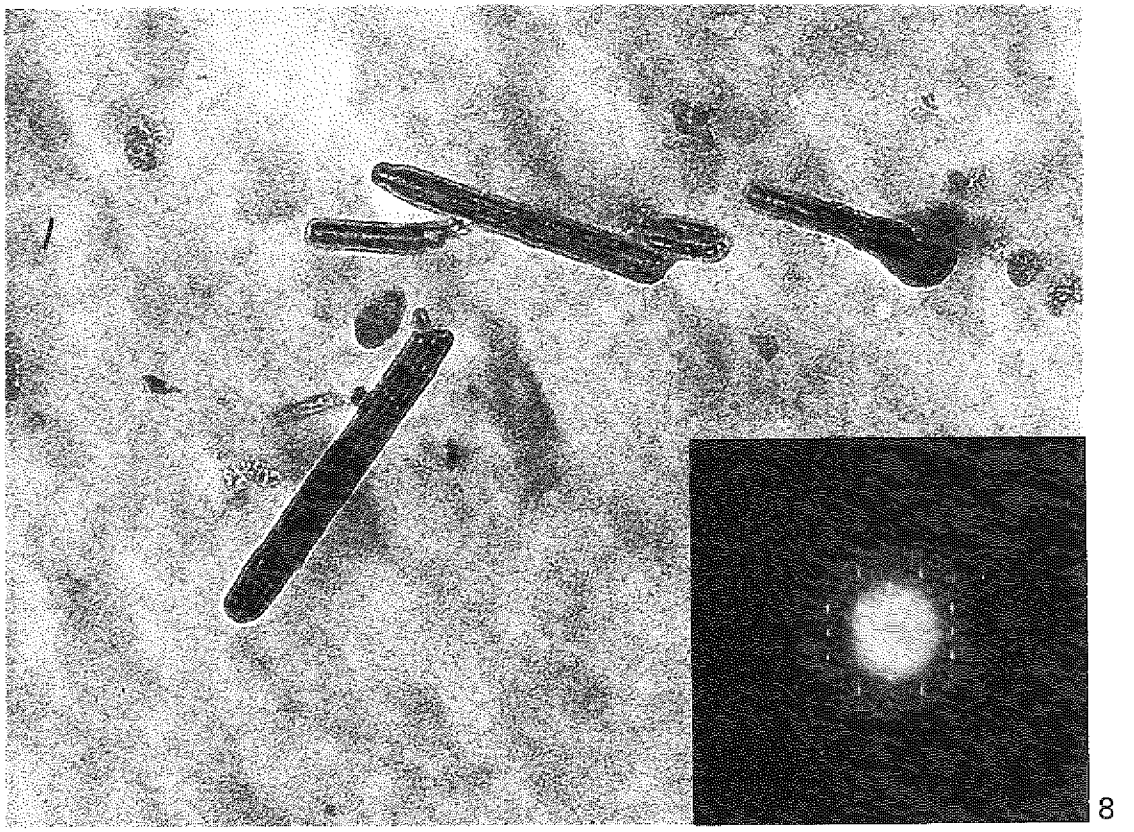

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Fig. 8. Parietal pleural plaque—calcified zone—chrysotile fibres visible after treatment with phosphotungstic acid and trypsin.

Table 1. Collagen level (in terms of decalcified tissue)

Nature of specimen	Collagen %
Calcified pleural plaque calcified zone fibrous zone	66.0 69.4
Fibrohyaline plaque (patient not exposed to asbestos dust)	65.7

of collagen in the fibrohyaline plaques is identical to that in the calcified plaques.

Phosphorus and calcium

These elements were measured by colorimetry and atomic absorption, respectively. The results are presented in Table 2.

Table 2. Phosphorus and calcium content

Nature of specimen	P %	Ca %	P : Ca
Calcified pleural plaques A — calcified zone — fibrous zone B — calcified zone — fibrous zone	6.90 0.08 6.30 0.51	26.80 0.14 27.80 1.40	0.25 0.57 0.23 0.36
Fibrohyaline plaque (subject not exposed to asbestos dust)	0.25	0.62	0.41

If the P : Ca values shown in the table are compared with those of natural calcium phosphates of the whitlockite or apatite types (0.51 and 0.46, respectively) they can be seen to deviate considerably from the stoichiometric values of those minerals, particularly in the case of the calcified fractions.

Hexosamines

Hexosamine levels were measured by colorimetry, using the method of Elson & Morgan as modified by Boas (1953). The results obtained are given in Table 3.

Table 3. Hexosamine level (in terms of decalcified tissue)

Nature of specimen	Hexosamines (in μM/g)
Calcified pleural plaque calcified zone fibrous zone	33 22
Fibrohyaline plaque (subject not exposed to asbestos dust)	19

It will be seen that the hexosamine level is markedly higher in the calcified than in the fibrohyaline fractions. This result must be compared with those obtained histologically by specific staining methods. Although they are stained very easily by the Van Gieson stain, fibrohyaline plaques take little stain from PAS and alcian blue; however, after decalcification, those zones which were previously rich in calcium compounds are impregnated more strongly by the two dyes, indicating their greater abundance of mucopolysaccharides, particularly of the acidic type.

Treatment of decalcified sections with hyaluronidase led to an almost total disappearance of the differences in staining between the calcified and fibrous zones, thus confirming the higher concentration of certain acidic mucopolysaccharides (hyaluronic acid and/or chondroitin sulphates A and C) in the calcifications.

PRESENCE OF ASBESTOS PARTICLES IN THE PLEURAL PLAQUES

The role of asbestos fibres in the pathogenesis of pleural plaques seen in persons exposed to asbestos dusts has never so far been successfully demonstrated. The demonstration of the presence of these fibres in the fibro-calcareous tissue of the plaques raises in itself a difficult technical problem.

In order to throw some light on the possible presence of asbestos fibres in the pleural plaques two sorts of examination were carried out:

(i) The plaques were first ashed in activated oxygen at 150°C. After moderate washing with dilute hydrochloric acid in order to eliminate the calcium phosphates, the ash was filtered onto a carbon membrane and examined under the electron microscope. The fibre concentrations measured in the lung and in the different parts of the pleural plaque were compared.

(ii) Thin sections were treated with a phosphotungstic acid solution to dissolve the apatite and whitlockite crystals and then subjected to trypsinisation. They were then examined for asbestos fibres.

These two methods demonstrated the presence of very fine mineral fibres in the pleural plaques (Figs. 7 & 8). The length of these fibres varied between 0.1 and 2 μm, and their diameter was of the order of 25–30 nm.

Electron diffraction showed that in the two cases

studied the fibres were those of chrysotile. In the sections treated with phosphotungstic acid and trypsin the fibres are surrounded by a thin sheath of an unidentified substance.

The results given in Table 4 show that pleural plaques found in persons who have been exposed to asbestos dusts may contain considerable quantities of very fine fibres whose presence can be demonstrated only after calcium deposits and collagen fibres have been eliminated.

Table 4. Results of asbestos fibre counts

Nature of specimen	No of fibres per cm³ of tissue	
	Case No 1	Case No 2
Lung tissue	10×10^6	1.6×10^8
Pleural plaque		
fibrous zone	6×10^6	3.6×10^8
calcified zone	30×10^6	40×10^8

Diameter of fibres : 25–30 nm
Length of fibres : 0.1–2 μm

DISCUSSION

The organisation of pleural plaques as seen in the light microscope has already been described and calls for no particular comment. On the other hand, infrastructural and biochemical study and electron diffraction techniques have thrown light on a certain number of points:

Calcifications

Microscopic nodular or acicular calcareous structures can be seen scattered throughout the fibrous part of calcified plaques as well as in fibrohyaline plaques. These structures are similar to those which form large areas of mineralisation in the calcified zones, thus indicating that these different tissues are related and essentially differ only in their degree of calcification.

Nascent calcifications are encountered in the fibrous zone mainly in the sectors which contain cell debris, or within the hyaline substance. In the calcified zone, the growth of the crystals is orientated in part by the collagen fibres.

The calcifications are essentially of the whitlockite and apatite type. Significant differences are found, however, in the phosphorus and calcium composition of these minerals, so that a more thorough study would appear to be necessary to clarify this point.

Organic matter

This consists essentially of fibres and of amorphous matter. The collagen fibres are of normal periodicity with a small proportion of wide diameter fibres.

Ultrastructurally, the amorphous matrix shows a microfilamentous organisation similar to that described for the ground substance of collagen tissue. Furthermore, biochemical analysis demonstrates the presence of hexosamines in greater quantities in the calcified zone than in the fibrous zone; and this is confirmed more specifically for the acidic mucopolysaccharides by histochemical methods.

Asbestos fibres

The presence of numerous asbestos fibres in the pleural plaques of persons exposed to asbestos dust constitutes an important finding. In the cases examined the fibres were all of very small size and hence completely invisible under the light microscope; moreover, their detection by electron microscopy requires a suitable technique. It should be noted that they are present in a much higher concentration in the calcified zone than in the fibrous zone.

Histogenesis of the pleural plaques

Data on this are still very incomplete and thus we are unable to sketch the probable process for the formation of pleural plaques.

It may, however, be noted in regard to the conditions under which fibrohyaline plaques appear that they are not the specific result of exposure to asbestos and that they can be observed in other circumstances.

On the other hand, it is interesting to note that the only pleural plaques showing massive calcifications contained large amounts of asbestos fibre, and that the calcified zone contained more abundant fibres than did the surrounding fibrous zones.

At a more basic level, the initiation of these calcifications and their shape seem to be dependent on the substrate on which their nucleus is formed.

To sum up, although the appearance of fibrohyaline tissues and of calcifications are both relatively common phenomena in general pathology, it may be assumed that the occurrence of these two processes together in this particular site suggests a definite relationship with the risk of asbestosis.

SUMMARY

A study has been made of the ultrastructure of fibrohyaline tissue and calcification in pleural plaques. The microscopic calcifications present in the fibrous zone of these plaques are comparable to those in the calcified zone which have the structure of whitlockite and apatite; however, their P:Ca ratio is different from that of those phosphates, particularly in the highly calcified zone. The level of hexosamines, and particularly of acidic mucopolysaccharides, is higher in the calcified zone than in the fibrous zone. Calcified pleural plaques from persons exposed to asbestos dust contain numerous fibres of that mineral.

ACKNOWLEDGEMENTS

The authors are indebted to C. Normand and G. Tichoux for their technical assistance.

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Immunology and asbestosis

M. TURNER WARWICK [1] in collaboration with R. Parkes, A. Hanson, W. Smither & P. Harries

INTRODUCTION

It is known that the majority of workers who develop intrapulmonary fibrosis have been heavily exposed to asbestos fibre for prolonged periods, but it is equally recognised that there are large numbers of workers who have had similar exposures who remain healthy. Many factors may be important in determining this divergence, including differences in macrophage handling of fibre within the lung, different fibrogenic responsiveness to fibre, or perhaps secondary events such as bacterial infection and secondary immunological factors which may lead to accelerated fibrosis.

This paper summarises some recent studies undertaken to identify and define some of these immunological host responses.

It is known that non-organ-specific autoantibodies are found in some forms of pulmonary fibrosis not apparently related to exogenous causes (e.g., cryptogenic fibrosing alveolitis). Further, certain tissue autoantibodies appear to modify pathological responses to certain inhaled inorganic dusts in the lung (Caplan, 1953). Their presence and their relation to clinical features in asbestosis might therefore be important.

METHODS

This report summarises some of the results obtained in three systematic surveys of asbestos workers exposed to different amounts of fibre under rather different conditions for contrasting lengths of time.

(1) *Pneumoconiosis Medical Panel, London (PMP)*

All subjects referred for pension assessment to this Panel between 1968 and 1972 were asked to attend the Brompton Hospital for standard physiological and immunological studies. Data was obtained on 196 subjects. The majority had abnormal chest radiograms.

(2) *East Ham series*

In collaboration with Dr Smither and Dr Hanson of the East Ham Chest Clinic, serum was collected from a series of workers who had been exposed to asbestos, mainly under defined factory conditions, during their employment, and who were undergoing long-term routine follow-up. Serum from 252 subjects has been collected between 1969 and 1971. The majority had normal or only slightly abnormal chest radiographs.

(3) *Medical Research Unit, Royal Naval Dockyard, Plymouth (MRU)*

In collaboration with Dr Harries and Dr Oldham, serum has been obtained from a series of naval personnel having direct or indirect exposure to asbestos during their employment ashore or afloat. Samples from 334 subjects were obtained between 1968 and 1971. The exact data from this series of subjects is only provisional because some discrepancies have been found between the hand-sorted questionnaires and the computer data which accounts for minor variations in the tables. These differences must be reconciled before a definitive analysis can be made. The majority had virtually normal chest radiographs.

A simple standard questionnaire was completed on each subject in each of the three series. This related to duration of exposure, dates of exposure, nature of employment, symptoms, physical signs and radiographic appearances. The probable but only approximate intensity of exposure was defined in terms of the nature of the job undertaken for the

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majority of their employment (Harries, 1971). For instance, exposure was regarded as "heavy" for laggers, strippers, sprayers and those handling raw dry fibres; as "light" for those working in the vicinity but not handling asbestos, for wet-fibre workers and for those handling finished products or having occasional contact with asbestos out-of-doors. Those probably exposed to intermediate amounts were classified as "medium".

IMMUNOLOGY

Non-organ-specific autoantibodies. Antinuclear antibody (ANA) and rheumatoid factor (RF) were measured and titrated by standard methods previously described (Turner Warwick & Parkes, 1970).

Lung reactive autoantibodies were measured by complement fixation and by antiglobulin consumption titre (AGCT) (Burrell *et al.*, 1964; Turner Warwick & Haslam, 1971).

Lymphocyte sensitisation to nuclear antigens was measured *in vitro* by inhibition of leucocyte migration (Soberg & Bendixen, 1967).

Lymphocyte responses to amosite, chrysotile and crocidolyte were studied using lymphocyte transformation and inhibition of guinea pig macrophage migration. These responses were compared to those obtained using non-specific stimulation by phytohaemagglutinin (PHA).

Lung biopsies were studied by indirect immunofluorescence by the methods previously described (Turner Warwick *et al.*, 1970).

RESULTS

The ages and duration of exposure are compared in Table 1. The overall prevalence of ANA and RF in the three population surveys are shown in Table 2: ANA and RF are significantly higher in the PMP series.

Table 1. Age and duration of exposure to asbestos in three surveys

	PMP	East Ham	MRU
AGE			
Mean	54.7[a]	49.6	47.8
SD	9.2	14.1	
DURATION			
All exposures			
Mean	19.0[a]	12.5	14.8
SD	12.2	10.3	10.2
Heavy exposure only			
Mean	19.0[a]	12.0	
SD	12.4	10.6	
Those with abnormal physical signs			
Mean	17.5	18.3	
SD	10.4	9.2	

[a] Value for PMP significantly different from that for East Ham.

Table 2. The prevalence of antinuclear antibody and rheumatoid factor in three surveys of asbestos workers

	PMP (1967–72)		East Ham		MRU	
Totals	196		252		334	
Antinuclear antibody total	39	*20*[a]	19	*7.5*	28	*8.4*
Rheumatoid factor total	23	*11.7*	13	*5.3*	12	*3.6*
ANA alone	31	*15.8*	15	*5.8*	26	*7.7*
RF alone	15	*7.7*	9	*3.6*	10	*3.3*
ANA + RF	8	*4.1*	4	*1.6*	2	*0.6*

[a] Italic figures indicate percentages.

The relation between the prevalence of autoantibodies and the probable intensity of exposure is shown in Table 3. The relation between autoantibodies and the presence of abnormal signs (rales and finger clubbing) is given in Table 4; and the relation between intensity of exposure, the presence of physical signs and autoantibodies is shown in Table 5.

From this data it appears that the increased prevalence of autoantibodies, especially of ANA, is related to the presence of abnormal physical signs in subjects following heavy exposure to asbestos fibre; it is

Table 3. Exposure and antibodies

Exposure	PMP				East Ham				MRU			
	ANA		RF		ANA		RF		ANA		RF	
Heavy	30/134	*22.6*[a]	16/134	*11.9*	7/108	*6.5*	2/108	*1.8*	19/145	*13.2*	8/145	*5.5*
Medium	9/38	*23.6*	5/38	*13.2*	5/69	*7.2*	4/69	*5.8*	9/68	*13.2*	4/68	*5.9*
Light	0/15	*0*	1/15	*6.7*	7/64	*10.8*	4/64	*6.3*	8/155	*5.2*	6/155	*3.8*

[a] Italic figures indicate % positive.

Table 4. ANA and physical signs
All exposures

	PMP		East Ham		MRU	
Clubbing with and without rales	15/49	*30.6*[a]	3/29	*10.3*	8/31	*25.8*
Rales only	15/76	*19.8*	4/62	*6.4*	5/46	*10.9*
Neither	4/45	*8.9*	12/157	*7.7*	23/284	*8.1*

[a] Italic figures indicate % positive.

Table 5. ANA and physical signs in different exposure groups

	PMP		East Ham		MRU	
	Heavy exposure					
Clubbing with and without rales	13/43	*30.3*[a]	3/12	*25.0*	7/21	*33.3*
Rales only	13/55	*23.5*	1/19	*5.6*	2/29	*6.9*
Neither	2/27	*7.4*	3/75	*4.0*	10/93	*10.7*
	Medium and light exposure					
Clubbing with and without rales	2/6	*33.0*	0/17	*0.0*	1/10	*10.0*
Rales only	2/21	*9.5*	3/40	*7.5*	3/17	*17.6*
Neither	2/18	*11.5*	9/76	*11.8*	13/191	*6.8*

[a] Italic figures indicate % positive.

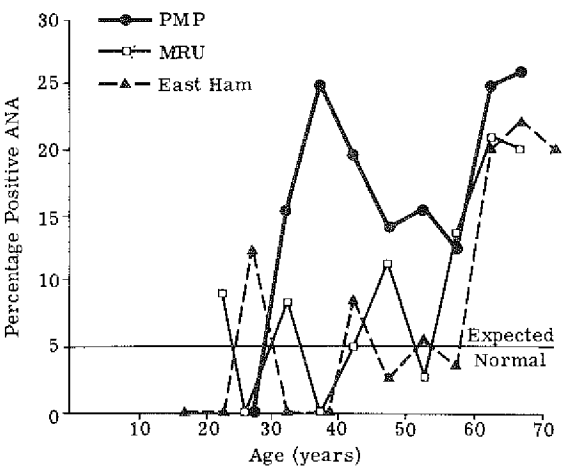

Fig. 1. Percentage positive ANA related to age in three surveys of asbestos workers.

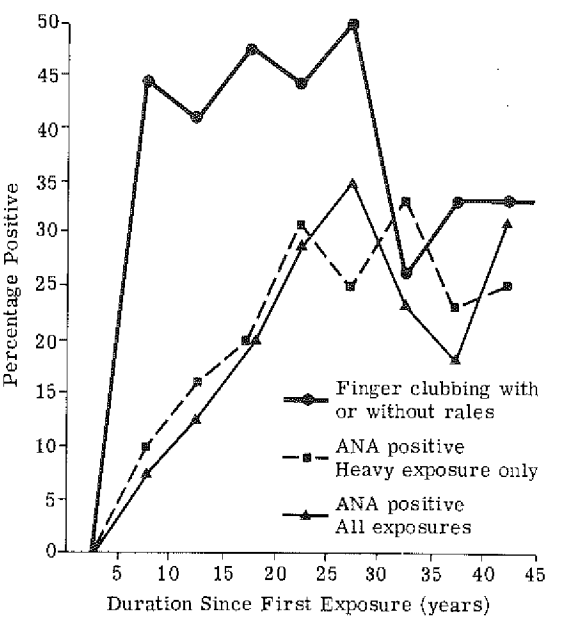

Fig. 2. Percentage of cases with physical signs, and percentage with ANA, related to duration since first exposure (PMP series).

seen less frequently in those with heavy exposure without abnormal physical signs.

Further analysis shows that when the percentage of positive ANA serum is related to age at the time of assessment, the PMP series differs from the other two in that here ANA shows a great increase in the younger age groups (Fig. 1). When the percentage frequency of ANA is plotted against the duration since first exposure in the PMP series (Fig. 2) there appears to be an almost linear relation up to 25–30 years, but no further increase thereafter. This relation is maintained when those with heavy exposure only are considered. The relation between this curve and the development of abnormal physical

signs (clubbing with and without rales, Fig. 2) appears to show a plateau at a similar point, about 25 years from first exposure.

By comparing the curves relating duration since first exposure to extent of radiographic change in ANA-positive and ANA-negative groups it should now be possible to obtain evidence as to whether ANA influences the course of disease or whether it arises as a consequence of fibrosis. Analysis of the first fifty-three unselected PMP cases (Fig. 3) shows

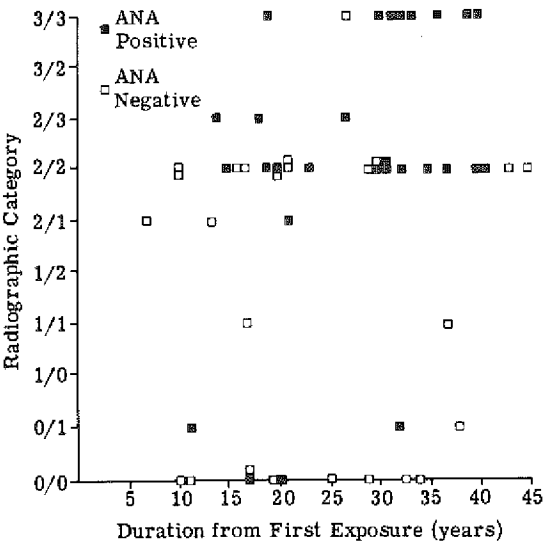

Fig. 3. Fifty-three unselected cases from PMP series only. The relation between the severity of radiographic abnormality and duration since first exposure in workers with and without antinuclear antibody.

that over a comparable range of times from first exposure, those with ANA had more advanced radiographic change compared to those without ANA. This distribution might be expected if ANA acted as an accelerator, after fibrosis had been initiated by a separate agent.

Lymphocyte sensitisation to nuclear antigens

In contrast to systemic lupus erythematosus (SLE) and fibrosing alveolitis, where the presence of ANA is associated with *in vitro* evidence of lymphocyte sensitisation to nuclear antigens, our study of lymphocytes from patients with asbestosis, when viewed as a group, showed no evidence of lymphocyte sensitisation to these antigens. See Table 6 (Haslam & Turner Warwick [1]). Individual subjects showed evidence of slight lymphocyte sensitisation to individual antigens.

Table 6. Lymphocyte sensitisation
% Inhibition leucocyte migration

	DNA 100 µg/ml	Nuclei 100 µg/ml	Nucleoprotein 6.35 µg/ml
Controls	7.1 (2.1)[a]	7.9 (4.0)	7.9 (4.2)
Asbestosis	5.0 (4.7)	13.9 (7.2)	7.3 (5.2)
SLE	*24.7*[b] (8.7)	*29.5* (5.8)	*27.2* (7.4)
Fibrosing alveolitis	15.7 (7.3)	*26.9* (5.9)	6.8 (5.7)

[a] Figures in parenthesis indicate standard error of mean.
[b] Italic figures indicate significant difference from controls.

Lung reactive antibodies

Serum from the PMP series including those with and without non-organ-specific autoantibodies was tested. Antiglobulin consumption test (AGCT) was positive in only 2/22 (9.1%) and complement fixation tests were positive in only 3/73 (4.1%). The two sera found positive by AGCT were also positive by complement fixation. The development of lung reactive antibodies does not seem to be important in asbestosis (Turner Warwick & Haslam, 1971).

Lymphocyte studies using asbestos fibre

Preliminary studies suggest that cytotoxicity rather than immunological lymphocyte sensitisation accounts for the majority of our results in both lymphocyte transformation and the inhibition of guinea-pig macrophage migration (Maini & Turner Warwick [2]).

Lung biopsies

Occasional intra-alveolar macrophages have been seen to contain immunoglobulin M (IgM), and also apparently to contain complement. No other evidence of complex deposits was found in alveolar walls, and no evidence of immunoglobulin-containing plasma cells was seen (Turner Warwick *et al.*, 1971).

COMMENTS AND CONCLUSIONS

The definitions of intensity of exposure in our context are necessarily somewhat arbitrary and imprecise. Usually one man undertook many different

[1] Unpublished data.
[2] Unpublished data.

types of jobs involving various exposures to asbestos; and apparently a job executed under controlled factory conditions resulted in far less exposure than when the same job was conducted in the uncontrolled outside environment. The exact exposure of workers afloat is likely to have varied considerably. Those doing a job in early years may have been exposed to far greater amounts of fibre than those undertaking the same job under improved conditions in later years.

These difficulties are well recognised and are certainly likely to be an important influence in the analysis of data from the three surveys reported here.

Many interpretations may be given to the data presented, and some points will certainly be clarified further after completion of the total computer analysis. This complete analysis has been delayed until all three surveys are complete and until the PMP series is large enough to be usefully comparable.

SUMMARY

With the foregoing provisos, the following conclusions are presented:

(*a*) Non-organ-specific autoantibodies, especially ANA, are found with increased frequency in those asbestos workers with abnormal physical signs suggesting asbestosis, who have been heavily exposed to asbestos dust for long periods of time.

(*b*) In contrast, the amounts of autoantibodies are only slightly increased in those with heavy exposure but without lung disease.

(*c*) In the PMP series there is an almost linear relation between the percentage of positive-ANA and duration from the time of first exposure, with a plateau at 25–30 years.

(*d*) A trend is shown in all three surveys of a slightly higher than expected prevalence of ANA in workers without clinical signs, and this may suggest that there is an early stimulus to ANA production in some asbestos workers.

(*e*) Lymphocyte sensitisation to nuclear antigens was not found in asbestosis.

(*f*) No evidence of lung-specific antibodies has been obtained.

(*g*) Challenge of lymphocytes by asbestos fibre results mainly in cytotoxicity.

(*h*) Occasional intra-alveolar macrophages appear to contain immunoglobulins, mainly IgM and complement. Whether this occurrence relates to an immunological response to asbestos fibre or to coincidental microbial infection has not been ascertained.

(*i*) Preliminary analysis supports the suggestion that ANA acts as an accelerator once the fibrosis has been initiated by a separate agent.

ACKNOWLEDGEMENTS

Population studies are essentially teamwork. I should like to acknowledge the most friendly collaboration I have received from Dr Audrey Hanson, Dr Walter Smither, Dr Peter Harries, Dr Oldham and Dr Raymond Parkes. I am grateful to my research assistants, Mrs Weeks, Mrs Haslam and Miss Anna Lukoszek for technical help. I am also grateful to the Asbestos Research Council and to the Chest and Heart Association who supported part of this work.

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Discussion summary

I. WEBSTER [1]

At the opening of the discussion, the importance of which methods were used to examine tissues and of the residence time of asbestos in the lung were emphasised. It was suggested that the studies reported by Dr Oldham should be followed by one in children.

The observation of Ashcroft and Heppleston that no relationship could be found between the degree of severity of asbestosis and residence time of asbestos in the lung in a small group of cases of asbestosis may indicate a limitation of the concept of "duration" of exposure. Many factors may affect the progression of pulmonary fibrosis, and variations in host response have to be considered, as well as infection which may occur before severe asbestosis develops.

Contrary to the evidence given by Davis and Gross, Dr Mancuso was of the opinion that short asbestos fibres contributed to the fibrosis but were difficult to measure in air samples. They also probably played a significant role in the development of malignant disease of the respiratory tract.

Dr Navratil suggested that calcified pleural plaques would either remain as static areas on the pleura or progress through an acute reaction. He did not consider that they underwent malignant change.

Dr Sluis-Cremer presented a study of a survey of asbestos cement workers in South Africa, showing that out of 1400 workers 180 had uncalcified pleural plaques. Although it was considered that pleural plaques were of no clinical significance, there was insufficient time to discuss the possible role of these plaques in the development of mesothelioma of the pleura. The rheumatoid factor, as detected by the HEA test, was positive in a significant number of employees showing plaque formation. Although the development of plaques was dose-response related, they occurred in a particular group of workers, possibly indicating a susceptible population.

There was also a report by Mr Gibbs on 520 cases of pleural plaques found in a survey of 15,000 X-ray plates in Quebec. An increased incidence of these plaques was found in workers in underground mines and in the maintenance staff, and appeared to be related to the inhalation of the dust from the surrounding rock rather than of asbestos fibre. The pleural plaques occurred in the Thetford area and not in Asbestos, and were associated with mining a talc carbonate layer present only at Thetford.

Dr Governa described work on the immunology of asbestosis. Lymphocytic infiltration occurred in animals and in man and could be related to the antigenic properties of coated asbestos fibres. As test systems, asbestos fibres coated with phytohaemagglutinin (PHA) or bovine serum albumin (BSA) were used; the degree of lymphocyte response was determined by using tritiated thymidine. Both PHA and BSA when combined with chrysotile reacted with lymphocytes. Other cultures with increased amounts of PHA and BSA did not react. Splenic cells gave a negative reaction.

[1] National Research Institute for Occupational Diseases, Johannesburg, South Africa.

A REVIEW OF CLINICAL DATA IN MESOTHELIOMA

Chairman — F. Gregoire

Rapporteur — G. Green

The natural history of diffuse mesothelioma

P. C. ELMES [1]

This paper gives a description of the clinical features and course of diffuse mesothelioma of the pleura and peritoneum. No attempt is made to distinguish those cases in which exposure to asbestos may have been a contributory cause of the disease from those cases where there was no evidence of exposure. The description has been limited to those cases where there is a consensus of opinion among pathologists as to the diagnosis. Generally, this has meant that the diagnosis has been confirmed by the exclusion of other primary neoplasms at autopsy. Because of diagnostic doubt, many of the early case reports have had to be set on one side, although reports such as those of Robertson (1924) and Hochberg (1950) contain sufficient detail to allow the inclusion of some of their cases. Since the suggestion by Wagner *et al.* (1960) that mesotheliomas were related to exposure to asbestos, there has been a large number of reports concerned with histological diagnosis and asbestos exposure. In many of these papers the clinical details were lacking, just as in the earlier literature the pathological diagnosis was not clearly established. That errors can arise from indiscriminate use of published material has been emphasised by the retrospective diagnostic studies of Newhouse & Wagner (1969).

In assembling the composite picture, I have relied on the following groups of cases (placed in order according to the amount of information available):

(1) A group of 63 cases studied by myself in Northern Ireland. In each case the diagnosis has been agreed by the London Mesothelioma Panel. Many of these cases were seen during their lives by me, and I have had access to all the notes and to the autopsy findings. This group was briefly reported at a meeting in Dublin in September 1971 (Elmes, 1972).

(2) A group of 111 cases from the rest of Great Britain. These represent part of a study of all the cases accepted by the London Mesothelioma Panel who died between January 1960 and December 1969. In these cases I have had access to the clinical notes and autopsy findings. It is my intention to study all the cases accepted by the Panel over this period to obtain a more accurate picture of the natural history of mesothelioma in the British Isles.

(3) Some 118 cases gleaned from the literature in which the clinical descriptions contain sufficient detail for analysis and which appear to have been based on sound pathological diagnosis. To avoid duplication, all cases from Britain were excluded.

(4) Other case descriptions and papers published in the world literature.

Naturally, I must have missed or excluded many genuine cases of mesothelioma. However, the uniformity which emerges from the descriptions which I have read indicates that the picture is reasonably complete.

AGE, SITE AND SEX

The Northern Ireland group

All the cases were men (although two female cases have occurred, they both died after 1 January 1970). The age at death is given in Table 1. There is a wide range of ages, including cases who first developed symptoms in their late 70's and 80's. It is probable that some of the older cases would not have been picked up in a study of a working population, as the follow-up would not have been continued so late. This gives a mean age of onset of symptoms of 62 years, which is rather older than in most reported groups.

[1] Whitla Professor of Therapeutics and Pharmacology, The Queen's University of Belfast, Institute of Clinical Science, Belfast, UK.

Table 1. Number and ages of cases

	Number of cases				Age at death		
	Total	% Male	% Pleural	% Right	Mean	Range	SD
Northern Ireland	63	100	97	65	63.3	39–88	11.3
Other British	111	78	71	50	60.3[a]	32–82	9.4
Other reported cases	121	85	91	—	54.25[b]	32–83	—

[a] For peritoneal cases only, 60.2 SD 9.11. [b] For peritoneal cases only, 61.6 SD 8.58.

Other cases

There were 111 cases from Great Britain, and the average age at death was less (60.3 years) than that of the Northern Ireland cases. One hundred and eighteen cases from other countries were analysed, and in these the average age at death was lower still, at 54.25 years. Although these differences may be statistically significant, they are likely to be due to selection. The general reluctance to pursue diagnosis in the elderly is liable to diminish the frequency of case recognition in this age group, except where special efforts are made.

It seems unlikely that one side of the chest is really affected more frequently than the other, although Hochberg (1950) reported six right-sided cases out of seven. Stumphius (1969) includes 13 left- and seven right-sided lesions. In the Northern Ireland series, 65% were right-sided; but in the equally unselected cases from Great Britain, the numbers were equal.

Peritoneal lesions were less frequent than pleural in almost all series; and, allowing for doubt as to the primary site in some cases, it seems likely that less than 10% start in the peritoneum (Table 1). Enticknap & Smither (1964) reported a series of peritoneal cases from the London area, and it is these that are responsible for the relatively high proportion of peritoneal cases in Great Britain. As yet, there is no explanation for this difference.

MODE OF ONSET

Northern Ireland series (See Table 2)

In a population where cough and breathlessness are common symptoms in the elderly because of the prevalence of chronic bronchitis, it was often difficult to determine the onset of the illness. The development of pain was the most usual clear indication of the onset, but most patients also complained of breathlessness, cough, lassitude and weight loss by the time they were referred to hospital. The onset was usually insidious; the pain started as a dull ache or heaviness, becoming a real pain and interfering with sleep only after several weeks. Breathlessness was usually gradual in onset, even in cases where a large volume of fluid had accumulated. The gradual development of symptoms in an elderly population already used to subjective illness probably explains the delay between the onset of symptoms and the time at which they sought expert advice. The average delay was four months, and there was little evidence that this was due to a failure on the part of the primary medical advisor to recognise the serious nature of the illness. Although these Northern Ireland cases were all primary pleural tumours, in four cases the pain at the onset was referred to the abdomen; more frequently it was referred to the shoulder. The pain appeared to be due to infiltration of the chest wall rather than to friction between the pleural surfaces, as it was seldom related to breathing.

Table 2. Mode of onset in Northern Ireland—63 cases

		No.	(%)
Pain		36	(57)
Chest or shoulder	32		
Abdomen	4		
Breathlessness		27	(43)
Weight loss		8	(13)
Lassitude		7	(11)
Cough		3	(5)

Most of the pleural cases from Great Britain and in the world literature presented in a similar way. In a few, the sudden onset of breathlessness was found to be due to a large haemorrhagic effusion, sometimes complicated by a pneumothorax. There were also cases in all four groups in which a chest injury, with broken ribs and perhaps a haemothorax

(Hochberg, 1950) or an acute pneumonia, preceded the recognition of the tumour (Mann *et al.*, 1966; Tayot *et al.*, 1966; Huguenin-Dumittan, 1968; Stumphius, 1969). It is not possible from the information available to verify that the tumour was already present at the time of injury or infection; however, these patients failed to recover their normal health, and re-investigation after a few weeks or months revealed the tumour.

In other cases, there was no evidence of a definite precancerous state. Before the onset of symptoms most of the patients were perfectly fit and many had normal chest X-rays. Cases which were found as a result of a follow-up of workers who were heavily exposed to asbestos showed the pleural plaques and parenchymal changes one would expect to see in such populations. Where there was prolonged exposure there was sometimes a history of transient pleurisy or effusion (Eisenstadt, 1965). Such apparently benign illnesses have been reported in other people who have been followed for 10 or more years without serious sequelae (Harries, 1971).

The mode of onset of peritoneal tumours was almost invariably a dull pain, followed within a few weeks by abdominal swelling, anorexia and weight loss. Many cases were admitted to hospital with subacute obstruction within a month of the onset of symptoms.

CLINICAL FINDINGS

In the majority of cases of *pleural mesothelioma* the patients appeared in good general health when they were first seen in hospital. There was a marked variation in the extent of the disease process at this stage, some patients presenting with minimal changes in the chest either on examination or X-ray. A summary of the findings in the Northern Ireland groups is given in Table 3. Study of the subsequent course of these cases and of the material from Great Britain indicated that much of the variation in findings was due to the different stage in the disease.

Cases presenting with a small effusion and no evidence of a tumour mass often had serous fluid; but this fluid contained few if any malignant cells, there was no chest deformity, finger clubbing or rise in the erythrocyte sedimentation rate or change in the differential white cell count. However, during the course of their illness these cases developed at some time a haemorrhagic effusion, thickening first of the parietal and then the visceral pleura (often with the formation of irregular tumour masses on the former), gross finger clubbing, a raised sedimentation rate and a polymorph leucocytosis. Some did not reach hospital until all these features were present.

Table 3. Initial finding—63 cases

Finding	No.	(%)
Effusion	46	(73)
Bloody 24		
Serous 22		
Solid mass (with or without effusion)	18	(29)
Bone involvement	None	—
Other changes in chest X-ray[a] :		
None	39	(60)
Plaques only	15	(25)
Parenchymal changes	6	(10)

[a] No satisfactory film available in three cases.

Finally, in many cases, the pleural surfaces became adherent (although at autopsy there was often one or more encysted collections of fluid surrounded by dense tumour tissue); and the pleural thickening extended around the lung to the hilum, with extension in along the interlobular fissures and out along the bronchi. In the early stages, sufficient fluid often accumulated so that the mediastinum was pushed towards the normal side; later the pleural surfaces adhered, thickened and contracted. This led to a constriction of the lung and a pulling up of the diaphragm, as well as immobilisation and constriction of the thoracic cage on that side. This contraction led to a kyphosis to the affected side, with a drooping shoulder and overlapping of the ribs (Stumphius, 1971; Sleggs *et al.*, 1961).

In some cases patients presented with a solid tumour, which gradually extended round the lung and produced the picture which has just been described. It is possible that these cases had passed through a phase with a free effusion before they sought medical care. It is also possible that in these cases the tumour had arisen, in a pleural cavity already obliterated by adhesions, from pre-existing disease (tuberculosis or asbestosis).

While clubbing of the fingers and toes may develop during the course of the illness in most of the patients, obvious pulmonary osteoarthropathy is uncommon. One of the Northern Ireland cases presented with pain in the right chest and a solid tumour in the right costophrenic angle posteriorly. While under investigation, he developed an acute arthropathy which subsided dramatically when most of the

tumour was removed surgically, but which recurred when the tumour grew again.

There was no other evidence that mesotheliomas have any humoral effects. Cachexia and inanition usually developed late, at a stage when there was an obvious physical cause for it, or when treatment with analgesics or cytotoxic agents was being pushed to the limit.

Peritoneal mesotheliomas usually manifest a moderate degree of abdominal distension with signs of some free fluid when they are first seen. Tumours may not be palpable until the fluid has been withdrawn, and even then they may not be obvious if there is obstruction with distended loops of gut. Both on rectal examination and on abdominal examination through a lax abdominal wall, irregular ill-defined masses may be felt. There is no clinical characteristic which distinguishes mesothelioma from other tumours with diffuse peritoneal spread, except evidence of past exposure to asbestos.

INVESTIGATIONS

Haematological and biochemical tests have little diagnostic value in this disease. As mentioned earlier, the sedimentation rate is often normal initially but becomes raised later, especially if there is a haemorrhagic effusion. In this situation there may be anaemia and some non-specific changes in the plasma proteins.

Radiology of the chest reveals the presence of fluid and perhaps of a pleural mass. In the later stages, bone involvement with disappearance of ribs in the most painful area of the chest wall may occur. Irregular masses of tumour may be seen on the inner surface of the rib cage after the fluid has been withdrawn, especially if some air is inserted into the pleural cavity. At this stage the visceral pleura may be thin, but later it thickens or becomes adherent to the parietal tumour. Ultimately, tomograms, or an over-penetrated film, will show compressed lung surrounded on all surfaces by a layer of tumour 2–3 cm thick.

Depending on the extent and type of asbestos exposure, there may be pleural or parenchymal changes related to this; but, in 65% of Northern Ireland (Table 3) and about half of the other British cases, no such changes were present. In the other reported series there was more variation depending on the source of cases.

CLINICAL COURSE

It is difficult to determine the natural course of the disease, because in most of the reported cases various diagnostic and therapeutic measures were taken which may have affected the course. These are discussed in my second paper.[1]

However, from those cases which were subjected to little interference during life or who presented first in a terminal state, it is possible to deduce the following: The tumour seldom metastasises early, and blood-borne metastases are unlikely to contribute significantly to the clinical picture. In pleural cases the progressive contraction of the affected side of the chest is complicated by extension into the chest wall, mediastinum, pericardium and diaphragm. The chest wall invasion involves nerves and bone early, and the main symptom throughout the illness is of pain in the distribution of the intercostal nerves. There is often anaesthesia or altered sensation in the skin of the chest wall and abdomen. Sometimes, involvement of the axillary nerve or lower part of the brachial plexus may mimic Pancoast's syndrome, even to the extent of interfering with the sympathetic innervation of the face and eye.

Involvement of the upper mediastinum does not often produce florid superior mediastinum obstruction with plethora of the head and neck in the way that primary carcinoma of the lung and Hodgkin's disease may do. However, the tumour may infiltrate around the pulmonary vessels and into the pericardium in such a way as to limit cardiac function. Paraplegia may develop without radiological involvement of the vertebrae, and may be due to the cutting-off of the blood supply to the cord.

Spread of the tumour to the pericardium and opposite pleura may occur a few weeks before death and can be detected by a friction on auscultation or by the presence of fluid.

Extension through the diaphragm is often followed by free spread throughout the peritoneal cavity with ascites. It is not uncommon for pleural mesotheliomas to be re-admitted to hospital because of symptoms of peritoneal malignancy, and some cases of peritoneal mesothelioma may be found to have a pleural lesion; thus, at autopsy it may be hard to say which was the primary site. One patient was investigated for right basal pleurisy and was found

[1] See p. 277 of this publication.

to have a small effusion which did not respond to antituberculous therapy. Although no malignant cells were found, and the needle biopsy was negative, the lesion was presumed to be malignant. Some months later he was referred to a surgical clinic because a right inguinal hernia appeared to have become encarcerated. An operation revealed mesothelial tumour in the hernial sac, and autopsy a few weeks later showed tumour extending from the right pleural cavity through the diaphragm, filling the peritoneum and extending into the hernial sac.

Primary peritoneal cases usually present with ascites and incipient intestinal obstruction, for which little can be done. The obstruction soon becomes complete, and surgical relief, if effective at all, is short-lived. The average duration of symptoms to death is six months (Enticknap & Smither, 1964), compared with just over a year for the pleural cases.

SUMMARY

The presentation and clinical course of 63 cases from Northern Ireland and 111 from Great Britain is analysed and compared with 118 cases reported in the literature. The tumour mainly affects men, with a wide age range averaging about 60 years. The tumour usually starts in the pleura, but a small proportion start in the peritoneum. In either case, pain is usually the first symptom, followed later by breathlessness and loss of weight. Although the onset is insidious, the time course of the disease is short, averaging 13–14 months for the pleural cases and six months for the peritoneal. Although the diagnosis can often be established in life from the characteristic picture of the pleural disease, the peritoneal cases are not easy to distinguish from other abdominal malignant tumours.

ACKNOWLEDGEMENTS

This material has been prepared with the help of Mrs M. Simpson whose work on the prevention of asbestosis is supported by the Government of Northern Ireland. Dr P. V. Pelnar of the Institute of Occupational and Environmental Health, Montreal helped by supplying reprints and abstracts of the literature.

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The value of a cancer register in the study of asbestos tumours

M. GREENBERG [1]

INTRODUCTION

Prior to 1966, the Pneumoconiosis Unit of the Medical Research Council had recorded about 200 cases of mesothelioma diagnosed in Britain in the previous 15 years in which the histological diagnosis had been confirmed by experts; but in that year the register was handed over to the Medical Branch of HM Factory Inspectorate (now the Medical Services Division, Department of Employment). The objects of the register, as stated in a memorandum of the Senior Medical Inspector's Advisory Panel (1968), were:

(i) to record the annual number of deaths from mesothelioma of the pleura and peritoneum associated with asbestos exposure;

(ii) to ascertain trends in the incidence rates;

(iii) to discover, if possible, tumours occurring without any exposure to known or suspected occupational causes; and

(iv) to provide part of the evidence on which preventive measures should be based.

It was decided to investigate for the years 1967–68 as fully as possible all those cases in England, Wales and Scotland where information was received that a diagnosis of malignant mesothelioma had been considered during life or *post mortem*. An interim account has been published (Lloyd Davies, 1970).

SOURCES OF INFORMATION

The Registrars General forwarded to the survey centre copies of (*a*) death certificates which included a diagnosis of mesothelioma of pleura and peritoneum, and (*b*) Cancer Bureaux Registration which included a diagnosis of malignant mesothelioma. Information on some cases was received from chest physicians, surgeons, pathologists and coroners.

The Pneumoconiosis Medical Panel was instrumental in notifying cases where a diagnosis of mesothelioma had been made on subjects receiving Industrial Injury Benefit under the National Insurance Act, 1946, or where coroners had forwarded thoracic viscera to them for expert opinion on subjects not receiving compensation.

The majority of cases were notified from two or more sources.

METHOD OF INVESTIGATION

A detailed questionnaire dealing with details of asbestos exposure and clinical history was completed by HM Medical Inspectors of Factories.

OCCUPATIONAL EXPOSURE HISTORY

Where possible, this was obtained by interviewing patients thought to have mesothelioma. Otherwise, widows, widowers and members of the family, employers and workmates were interviewed.

Of the 246 subjects with definite tumours, 167 (68%) had an ascertained occupational asbestos exposure history. Of the remainder (79) of subjects studied (including "undecided", "insufficient material" and "definitely not mesothelioma") 38% had an ascertained occupational asbestos exposure history.

[1] Employment Medical Advisory Service, Department of Employment, London, UK.

While the difference between the two groups is statistically significant, this would be invalidated if there were diagnostic bias, in that mesothelioma was recognised and diagnosed predominantly in industrial areas where asbestos is known to be heavily used. Then, even in the absence of a cause and effect relationship between mesothelioma and asbestos, subjects discovered to have mesothelial tumours would be found to have worked predominantly in asbestos-using occupations. The distribution of reported and confirmed cases is not uniform over the country, nor is it entirely related to population density. This applies both to mesotheliomas associated with definite occupational asbestos exposure, and to those without. For example, Merseyside, Clydeside and Greater London had an increased frequency of diagnosis of mesotheliomas, approximately four and five times the national experience, and greatly in excess of that for the less densely populated portion of the remainder of their regions.

Merseyside and Clydeside share two important characteristics, one is the presence of shipbuilding industries using large amounts of asbestos; the other is the presence of physicians, radiologists and pathologists with a heightened awareness of the existence of mesothelial tumour, as evidenced by recent publications (Whitwell & Rawcliffe, 1971; Ashcroft & Heppleston, 1970; McEwen *et al.*, 1970).

Greater London shares with other major conurbations possessing asbestos-using industries an incidence of mesothelioma in excess of the national rate. When the distribution of cases in the London area was plotted, while there was a heavy concentration in East London where the major asbestos-using factories are situated, only half the cases diagnosed were found to have had occupational asbestos exposure histories.

If this is held to imply not merely a greater awareness of the disease but also widespread contamination of Greater London with asbestos, then the question arises why this does not occur similarly for other great conurbations with a high frequency of occupationally associated mesotheliomas, and with, presumably, equally widespread environmental pollution. If the reduced incidence of cases of mesothelioma in parts of the country away from asbestos industry is due to cases being referred from rural districts to hospitals in the greater conurbations, then this would have had the effect of raising the numbers of non-asbestos-associated tumours in all of the great conurbations.

ASBESTOS TYPE EXPOSURE

The four main types of asbestos, while all carcinogenic in animals, have not been equally implicated in the causations of human tumours, insofar as epidemiological evidence can be relied on (Wagner *et al.*, 1971). It was not possible to determine a significant difference among subjects (who had definite occupational asbestos exposure and where the diagnosis was either definite mesothelioma, undecided, or definitely not mesothelioma) in terms of the type of asbestos to which they had been exposed.

Retrospective studies such as are constituted by this type of register cannot be expected to resolve the question either of the hazard of asbestos or of the relative hazards of asbestos types. Other than in asbestos mining, it is exceptional for workers to be exposed to one type of asbestos only in the course of a lifetime, and memory is fallible. For quantitative evidence of asbestos exposure and for type, a technique such as that elaborated by Gold (1968) would be invaluable and, in general, distinctly superior to anamnesis.

THE INCIDENCE OF "CORROBORATIVE EVIDENCE" OF ASBESTOS EXPOSURE IN MESOTHELIOMA CASES

The discovery of a history of occupational asbestos exposure was frequently confirmed by the presence of asbestosis, pleural plaques, asbestos bodies or asbestos fibres. Where a history of occupational asbestos exposure was not obtained, however, it was also possible to find asbestos bodies or pleural plaques.

It could be argued that with prior knowledge of the occupational history, the histologist might be more or less assiduous in his search for corroborative evidence. Similarly, a diagnosis of mesothelioma might act as a spur to detect corroborative evidence of asbestos exposure. In the absence of a definite occupational asbestos exposure in cases definitely not mesothelioma, corroborative evidence of asbestos exposure was never found; whereas in definite mesothelioma in the absence of an occupational asbestos exposure history, evidence of asbestos exposure was discovered.

The significance of this difference is open to doubt in view of the considerations already discussed. In this register bias could not be eliminated. In any future survey a technique such as that employed by

Gold, where quantitative and qualitative evidence of asbestos in "normal" and neoplastic tissue is sought, would appear to be the one of choice.

PROBLEMS OF "CONTROLS"

The Registrar General for England and Wales provided from 1 January 1968 onwards a "control" death certification with each copy of a death certificate registering a mesothelioma. The "control" was selected on a one for one basis among persons of the same sex and age, but not matched for area of residence.

Subjects with mesothelioma were more commonly referred to the coroner than were subjects in the control group, because of their association with asbestos exposure. Consequently there was greater precision in job definition, which served to identify the asbestos association; thus, a "fitter" would be further categorised as "dockyard", and the death certificate would contain the words "an industrial disease"; a "plumber" or a "joiner" would be categorised further as "shipyard". Death certificates also read "motor driver", or "lorry driver", mentioning asbestos exposure and attributing disease to occupation. In this mesothelioma group, "housewife" and "widow" also appear as occupations with the disease attributed to asbestos exposure. The inquest rate was much less in the control group, and there would be no cause to sharpen job definition. The control subjects were scattered at random over England and Wales, whereas the mesothelioma subjects were more circumscribed.

CONCLUSIONS

A register labelled "asbestos tumours" will pose more problems by its very existence than it can hope to solve, though by its sheer publicity value it will of course bring to light a number of cases that might otherwise have been overlooked.

To establish the true incidence of mesothelioma would require not only a review by a panel of expert pathologists of putative cases of mesothelioma, but also a review of a stratified sample of tumours that are readily confused with mesothelioma. If the whole were carried out by pathologists working "blind" insofar as knowledge of occupational history and autopsy findings were concerned, reporting in a standardised manner on agreed histological and histochemical features, the diagnosis subsequently being scored on a confidence basis, then a closer approximation to the true incidence of mesothelial tumours might be arrived at. It is unlikely that autopsy techniques could be standardised.

In 68% of all cases in this survey a definite occupational asbestos exposure history was obtained. To evaluate the significance of asbestos exposure it would be necessary to investigate by careful history-taking and by means of quantitative and qualitative estimations of asbestos fibre body load, both subjects with mesothelial tumours and a control population matched for age, sex, geography and smoking habits. Diagnostic criteria and quantitative techniques should be established for asbestosis, asbestos bodies and plaques. It will also be necessary to know the relative sizes of populations thought to have been at risk and those thought not to have been at risk. Until a scrupulously careful control study has been made, neither the incidence of mesothelial tumours in man nor the role of asbestos in their formation can be confidently determined.

A cancer register is no substitute for a prospective scientific survey. "A mortality study of asbestos workers" has a more scientific ring to it than does "a registry of asbestos tumours". The Employment Medical Advisory Service of the Department of Employment has thus started a survey of workers exposed to asbestos that will study both morbidity and mortality patterns of workers exposed to measured amounts of specific types of asbestos, whether they continue in the industry or leave. A better idea of the hazards of asbestos exposure (including neoplasms) at the sanitary levels set in implementing the Asbestos Regulations, 1969 will result from this study.

SUMMARY

A register of cases of mesothelioma occurring in the United Kingdom was set up: (1) to record the annual number of deaths from mesothelioma of the pleura and peritoneum associated with asbestos exposure; (ii) to ascertain trends in the incidence rates; (iii) to discover, if possible, tumours occurring without any exposure to

known or suspected occupational causes; and (iv) to provide part of the evidence on which preventive measures should be based.

Because of limited resources, a full investigation of cases was restricted to the years 1967 and 1968. The major value of the activity was to publicise the disease and to enhance the experience of a panel of pathologists.

It was not possible to determine whether the frequency of diagnosis of mesothelioma in persons exposed to asbestos in districts with asbestos-using industries was based on increased incidence due to asbestos or to increased regional awareness because of a preconceived association between tumour and asbestos.

A study of asbestos workers by the Employment Medical Advisory Service of the Department of Employment is in progress to achieve the objectives for which the register was originally constructed.

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Therapeutic openings in the treatment of mesothelioma

P. C. ELMES [1]

Just as there are many methods for the treatment of mesothelioma advocated in the literature, so there are also many comments on their inadequacy: there is no effective curative treatment. Therefore it is necessary to review the various methods of management which have been advocated and to assess as far as possible their advantages and disadvantages. It is also necessary to include in this review diagnostic measures, for two reasons. Firstly, an attempt at surgical treatment (excision of a thickened pleura) may be the means by which the diagnosis is established and may become the routine approach in cases where the diagnosis of pleural mesothelioma is considered. Secondly, diagnostic procedures such as open or even needle biopsy may lead to spread of the tumour. The review which follows makes use of the same groups of patients referred to in my preceding paper.[2]

OBSERVED RESULTS

There is no published series in which matched groups of patients have been subjected to a variety of treatments in a prospective study. Retrospective studies are no substitute.

The fallacies of a retrospective analysis are illustrated in the Northern Ireland series.[2] The relationship between the diagnostic measures used in these cases and their survival is given in Table 1. It will be noted that those cases where a minimal effort was made to establish the diagnosis survived the shortest time (8.4 months) from the time they were first seen at the hospital. Those on whom a needle biopsy was done survived longest (11.4 months), and those on whom an open biopsy was carried out survived 10.8 months. The differences are not statistically significant. The needle biopsies were done using a technique requiring the presence of free fluid in the pleural cavity, and therefore very advanced cases could not be managed in this way. As such advanced cases were not submitted to surgery either, they were concentrated in the group with minimal diagnostic management.

Table 1. Effect of diagnostic measures on survival in months from referral

		Mean
Tapping only, or no local diagnostic measures	(26 cases)	8.4 (SD 8.6)
Needle biopsy only	(8 cases)	11.4 (SD 5.9)
Open biopsy or attempted excision	(27 cases)	10.8 (SD 9.1)

The Northern Ireland cases showed that open surgical biopsy or attempted excision of the tumour carried with it the risk of seeding in the scar and involvement of the skin by tumour. The tumour deposit would sometimes ulcerate and was always painful. Local extension along the needle track also occurred frequently after needle biopsy, but this was relatively uncommon after simple tapping for fluid. The frequency of this complication increased with the length of survival, so that it is not possible to quote frequencies in relation to the three diagnostic procedures.

Therapeutic measures related to survival in the Northern Ireland series are given in Table 2. Surgical excision was not included in this analysis because it was attempted in only eight cases and was usually combined with cytotoxic therapy. There is no significant difference between the survival time for the four different treatment groups, and again

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[1] See p. 267 of this publication.

the reason for this may be due to the method of selection. Initially, patients with considerable pain, who also failed to respond well to analgesic therapy, were treated with palliative X-ray therapy, which was never used to prolong life. Later, radiotherapy was avoided because it seemed to cause the tumour to grow more rapidly through the chest wall and to involve the skin. This skin involvement was never seen except through a wound or needle puncture, or through an area of skin subjected to radiation.

Table 2. Effect of clinical management on survival in months from date of referral

		Mean
No treatment	(23 cases)	9.6 (SD 9.6)
Tapping only	(23 cases)	9.4 (SD 8.9)
Local cytotoxic agent or local radiotherapy	(11 cases)	11.4 (SD 5.9)
General cytotoxic therapy	(5 cases)	10.4 (SD 6.5)

Local cytotoxic or radioactive therapy was used palliatively to prevent the rapid reaccumulation of fluid and to avoid repeated tapping for breathlessness. However, it was sometimes followed by a systemic upset which would tip the balance in a critically ill patient. Spontaneous disappearance of fluid seemed to occur in most cases anyway sooner or later, and the effect of local therapy was seldom more than to reduce the frequency of tapping from once per week to once every two weeks.

General or systemic cytotoxic therapy was used in only five cases, with the aim of arresting the relentless progress of the tumour in the terminal stages. It was not effective in this nor in the relief of pain.

Thus, the way in which the Northern Ireland cases were selected prevents an assessment of value of treatment to prolong life.

AVAILABLE METHODS OF TREATMENT

Surgical excision

Hochberg (1950) published a report on a series of seven cases in which he described the disease as amenable to surgical treatment. However, two of his operated cases died in the immediate post-operative period and two others died of recurring tumour six and nine months after operation. The experience of Sleggs *et al.* (1961) is similar. Although cases are reported who survived after surgery, in most of these the period of follow-up was under three years. The remainder were described as localised tumours. There is no authenticated case of prolonged survival after excision of a tumour having the characteristics of a diffuse mesothelioma with free fluid.

There are two possible benefits from surgical excision:

(i) The prolongation of life (Merlier, 1971). As already indicated there is little evidence for this except in localised lesions.

(ii) The prevention of fluid accumulation. This is claimed by several authors (Merlier *et al.*, 1968; Hertzog & Toty, 1968), and study of the case notes of the English cases supports this. What is less clear is whether the risk of pleurectomy and its complications, such as wound pain, is worth the benefit gained from obliteration of the pleural cavity.

Mesothelioma, untreated, tends to be confined to the serous cavity where it originates and only gradually extends outwards to involve chest, pericardium, diaphragm, peritoneum, etc. Local lymph node involvement is not uncommon, but it is seldom of clinical importance. However, among the Northern Ireland, Great Britain and reported cases there are a number of cases of otherwise typical mesothelioma with blood-borne metastases. These metastases have been of clinical importance, giving rise to symptoms, and have appeared in the brain, pituitary, adrenals and elsewhere. As far as I can determine, this dissemination has occurred only in patients who have had operative treatment, radiation or cytotoxic therapy. If, on further analysis, this proves to be true, then it is a further argument against too active therapy of this tumour.

Local cytotoxic therapy

At an early stage in the disease there may be a small amount of fluid containing malignant cells in the pleural or peritoneal cavity and a focus of tumour probably somewhere on the parietal surface. Although malignant change may arise in a plaque, plaques are not invariably present. At this stage it is theoretically possible to destroy the entire growth with a cytotoxic agent such as cyclophosphamide, with a nitrogen mustard or with a radioactive suspension of gold or yttrium. This procedure was frequently carried out in the Northern Ireland and Great Britain series, and there is no report of a cure.

Other authors recommend this procedure for the relief of pain or to slow the rate of accumulation of fluid, but the results seem disappointing (Oels *et al.*, 1971; Choubrac, 1971). This treatment should be used before the growth has become too thick and the serous surfaces have become adherent and walled off areas of growth. The difficulty is to establish the diagnosis confidently at a stage when the growth might be expected to respond.

The instillation of a cytotoxic agent or talc into the pleural cavity at the end of an operation for stripping the pleura has been carried out to prevent local recurrence, especially in the scar. The case histories of such cases are hard to evaluate.

Radiotherapy

Judging by the lack of beneficial effect for pain in the Northern Ireland and British series, it is very unlikely that radiation could be used in an attempt to cure at any stage. When patients with pain as the main symptom were treated by radiotherapy, pain remained the main symptom afterwards.

There is also the possibility of enhanced spread of tumour. This has been observed in cases where tumour has grown through the chest wall only in the irradiated area. Some cases which have been irradiated are also among those with clinically significant widespread metastases.

Systemic cytotoxic drug therapy

This has been used alone or in conjunction with local cytotoxic therapy in both pleural and peritoneal cases. It is not recorded as being effective for the relief of pain, but in some cases a reduction in the rate of accumulation of fluid and an improvement in the patient's general health have been claimed. Again, it is difficult to evaluate these claims, especially in view of the way effusions may dry up spontaneously in the course of this illness. There is also the possibility that this form of therapy may lead to dissemination of the growth; but the cases analysed include too many receiving multiple therapy.

FUTURE TRENDS

Both the clinical course of the untreated tumour and its histological characteristics suggest that there is a considerable contribution by the body's defence mechanism both to the formation of the abnormal tissue and to the shaping of the clinical course. Two additional factors may support this view, namely the relatively frequent occurrence of chest injuries and infections at the onset of the clinical course of the illness; and, secondly, the possibility that surgery, radiation and cytotoxic drug therapy may promote more rapid growth and dissemination. Injury, acute infection, operation, radiation and cytotoxic therapy all interfere with the body's reaction to invasion, partly directly and partly by provoking the excretion of cortisol from the adrenals. Surgery, radiation and cytotoxic therapy could only be expected to help these patients if the tumour cells were entirely destroyed—partial destruction might do more harm than good. On the other hand, measures to promote the body's reaction to the tumour might slow down the progress of the disease or halt it at a stage when it causes negligible symptoms. Such therapy would depend on very early diagnosis, before the tumour produces symptoms; at this point local cytotoxic drug therapy might be effective, or a provocation of tumour antibodies might arrest the tumour growth. At the present time, the only likely method of early diagnosis would be by the detection of tumour antibodies. Treatment might be by the use of tumour antigen to accelerate the body's reaction to the tumour. Both diagnosis and treatment would be dependent on the elucidation of the immunology of this tumour.

In the meantime, it seems that the best management of mesotheliomas of the pleura and peritoneum is symptomatic: to relieve pain with simple analgesics, reserving narcotics and nerve-root section for the later stages of the disease. Diagnostic measures should be kept to a minimum. Simple removal of fluid for cytologic examination and to relieve breathlessness should be carried out as infrequently and with as little trauma to the chest wall as possible. In the absence of a satisfactory curative treatment, the establishment of a precise diagnosis during life becomes an academic exercise. Only when another, treatable, disease is expected should open thoracotomy, surgical excision or possibly even needle biopsy be used. The introduction of cytotoxic agents or talc into the pleural cavity is probably of no value; and radiation, radical surgery and systemic cytotoxic drug therapy may be actively harmful. This is a disease in which the prevention is far more effective than the cure.

SUMMARY

Analysis of cases from Northern Ireland and Great Britain reveals that although many forms of treatment were used none were curative. In reviewing the value of the various forms of palliative treatment that have been advocated, the dangers of disseminating the tumour are noted. The possibility of treatment of very early cases by provoking antibody therapy is discussed. Until such treatment becomes available, the minimum diagnostic effort is recommended, followed by analgesics and pleural tapping.

ACKNOWLEDGEMENTS

This material has been prepared with the help of Mrs M. Simpson whose work on the prevention of asbestosis is supported by the Government of Northern Ireland. Dr P. V. Pelnar of the Institute of Occupational and Environmental Health, Montreal helped by supplying reprints and abstracts of the literature.

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Discussion summary

G. GREEN [1]

Dr Whitwell opened the discussion by pointing out the close affinity of the Northern Ireland mesotheliomas to those from Merseyside. These centres, on opposite sides of the Irish Sea, both have long-established shipyards, similar populations in numbers and ethnology, and have found similar numbers of mesotheliomas. The mean ages of patients at the onset of symptoms differed between the two groups only by two years, and the mean interval from first asbestos exposure to appearance of mesothelioma symptoms is almost identical. Symptoms and signs are similar. The duration of survival from first symptoms to death was 13–14 months in the Northern Ireland series described by Prof Elmes, and in Merseyside 21 months. There was no great difference in the survival rates of the different histological types. In contrast to Prof Elmes' thoughts on the effects of vigorous treatment on distant metastases, Dr Whitwell showed that four of eight patients who had received no more energetic treatment than pleural aspiration showed distant metastases *post mortem*. Of eight patients who had been treated more actively with surgery and had come to necropsy, only one had shown distant metastases. It did not appear that surgery stimulated metastases in these cases.

It was noted that in Merseyside mesotheliomas in women were seen only with definite occupational histories of exposure to asbestos dust. It was felt that criteria for a stricter definition of environmental exposure in cases with mesothelioma were required. Although evidence of occupational and domestic contamination was usually sufficient, there were obviously occasions in both these types of exposure where the total association had been so transitory that it may have been more correct to consider the exposure as environmental. Conversely, a family which had lived in the immediate vicinity of a site from which there had been a heavy discharge of asbestos dust could be classed as environmentally exposed but had received a far greater dose than those people working on the fringe of the industry. Probably the only reliable method would be to establish a dose-response relationship based on the analysis of dust in the lungs of people who had various types of exposure. As had been mentioned at previous sessions, the methods required to identify the type and to measure the quantity of the small amounts of asbestos in tissue are highly sophisticated. Nevertheless this type of study is urgently needed. An additional approach was being undertaken in South Africa where acute dust exposure records had been computed in retrospect for about 1000 people exposed to dust on the asbestos mines. It was pointed out that in some of the cases categorised as environmental exposures familial factors had been suggested.

The significance of pleural plaques was queried; whereas in the cases reviewed from the United Kingdom pleural plaques gave a poor prognosis as their presence was frequently followed by the development of mesotheliomas, in Finland no mesotheliomas had been associated with pleural plaques, showing that the type of asbestos fibre involved was important.

There was support for Prof Elmes' contention that a provisional diagnosis of mesothelioma was acceptable during life in order to avoid the potential complications of vigorous diagnostic procedures. If any effective therapy could be provided the diagnosis during life became important, and diagnostic procedures such as open thoracotomy would be justified. In the meanwhile, it was most important that post-mortem examinations should be carried out in these cases so that an accurate diagnosis using the

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UICC standard criteria could be made. This was essential for national registers and for correlation with epidemiological studies.

The question of humoral effects from the presence of mesotheliomas was raised, and Lowbeer's review of hypoglycaemia-producing extra-pancreatic neoplasms (Lowbeer, 1961) was mentioned. It was agreed that in this and subsequent papers it had been shown that the effect had been seen in cases with pleural neoplasms, usually solitary fibromas, but also in some cases with sarcomatous tumours. None of the delegates present had noted evidence of hypoglycaemia in the diffuse mesotheliomas that they had treated, and there was no recorded evidence of this association.

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CONSIDERATIONS OF ETIOLOGICAL MECHANISMS AND OTHER FACTORS

Chairman — A. C. Allison

Rapporteur — A. Morgan

Information obtained from animal experiments

J. C. WAGNER[1] & G. BERRY[1]

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 & Berry[2] 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, 1972[3]).

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 1.25×3.75 μm, could be discounted. The apparent disagreement on the significance of fibre size is discussed in the following two papers (Stanton [1]; Timbrell [2]).

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 (1972 [3]) in 17 out of 25 rats and 23 out of 50 mice injected with crocidolite.

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 Vôsamäe (1972). Miller and colleagues used hamsters, and Vôsamäe 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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[1] See p. 289 of this publication.
[2] See p. 295 of this publication.
[3] Personal communication.

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 (Stanton [1]; Timbrell [2]).

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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Some etiological considerations of fibre carcinogenesis

M. F. STANTON [1]

The exogenous agents which contribute to the cause of cancer generally fall into one of three major groups: ionising radiation, chemicals and viruses. There is a wealth of speculation as to how the members of these groups act to induce cancer, but the mechanisms of their action remain unknown. Asbestos, in all its forms, contains chemicals that are carcinogenic under certain conditions; however, it is of particular interest as a carcinogen because it has attributes of two of the above groups. Firstly, the presence of various metallic ions and polycyclic hydrocarbons, that are either inherent or acquired through processing, would seem the best explanation for the carcinogenicity of asbestos. On the other hand, particles that are within the dimensional range of viruses are abundant in all forms of asbestos; and it is conceivable that these submicroscopic particles could act in a fashion similar to that of viruses, whatever that may be.

On the other hand, there is reasonably good evidence that neither of these attributes is related to the carcinogenicity of asbestos. The evidence for this conclusion can be summarised as follows:

(1) There is no indication that any of the asbestoses are sufficiently contaminated with known carcinogenic hydrocarbons to account for their carcinogenicity; and rigorous extraction of those hydrocarbons present in asbestos does not affect its carcinogenicity for the pleura of the rat (Wagner *et al.*, 1970).

(2) Variations in the inherent metal content of various types of asbestos are great, yet these various types of asbestos show only slight differences in carcinogenicity (Harington, 1965; Timbrell, 1970; Wagner, 1970; Stanton & Wrench, 1972).

(3) Finely particulate metallic nickel, stainless steel or non-crystalline silicon dioxide applied to the pleura of the rat are not sufficiently carcinogenic to account for the carcinogenicity of asbestos by mill contamination (Stanton & Wrench, 1972).

(4) Reduction of fibre size by the partial pulverisation of asbestos, a process which increases contamination by metallic particles and increases the number of submicroscopic fibrils in asbestos, reduces its carcinogenicity (Stanton & Wrench, 1972).

(5) Hand-cobbed crocidolite ore, hand-milled without metallic contamination, is equal in carcinogenicity to machine-milled crocidolite (Stanton & Wrench, 1972).

(6) Non-asbestiform fibres such as fibrous glass are increasingly carcinogenic as they approach the size range of milled asbestos fibres (Stanton & Wrench, 1972).

One must therefore consider that it is the structural features of asbestos that may be the critical factor in its carcinogenicity, and it is toward this hypothesis that we have directed our attention. If the structural features of asbestos are important, then it follows that similar fibres, if sufficiently durable, should also induce tumours. On the basis of this reasoning, we have assessed the distribution of particles by size in a variety of fibrous materials and applied these to the pleura of rats for a period of two years. These experiments are still in progress; only preliminary results of part of them are available and interpretations are limited. However, estimates of final tumour incidences have been made from the data currently at hand, and the various materials have been segregated into four groups which cause high, moderate, low and negligible tumour incidence (Tables 1–4). The analysis of fibre distribution by size is subject to some error, nevertheless it is sufficient to characterise the general distribution of fibres in the materials.

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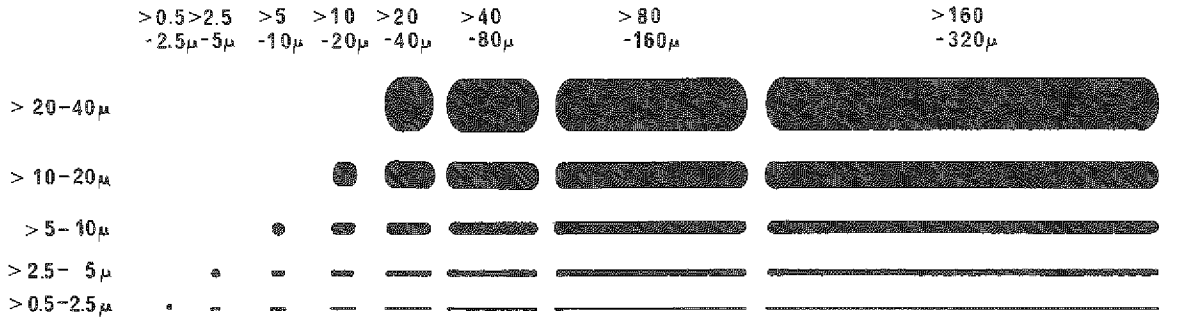

Fig. 1. Graphic illustration of the categories of size used to classify particles in the test samples. Illustrated particles represent mean dimensions (μ = μm).

MATERIALS AND METHODS

Various specimens of crocidolite, chrysotile, fibrous glass, and fibrous aluminium oxide were applied by open thoracotomy to the left pleural surface of 30, 11- to 14-week-old, female Osborne-Mendel rats at a single standard 40 mg dose level by a method previously described (Stanton *et al.*, 1969). The unique aspect of these experiments is that all test materials were applied to small 45 mg fibrous glass pledgets prior to application. The glass pledgets are composed of large-diameter fibrous glass which, when intact, has no apparent carcinogenicity in itself. We use it simply as a convenient and accurate means of uniformly applying the test material to a wide surface area of the pleura. The test materials are listed in Tables 1–4. The UICC standard reference samples of crocidolite and chrysotile A have been previously described (Timbrell, 1970). Reduction of particle size was accomplished by grinding in a stainless-steel

Table 1. High incidence groups (> 40% mesotheliomas). Percentage of mass occupied by fibres in each range of size

Diameter μm	Length μm							
	> 0.5–2.5	> 2.5–5	> 5–10	> 10–20	> 20–40	> 40–80	> 80–160	> 160–320
> 20–40								
> 10–20	Crocidolite UICC							
> 5–10	+ + + + [a]		1			11	22	
> 2.5–5		1	< 1	1	3	3	17	11
> 0.5–2.5	3	5	7	4	4	2	1	4
> 20–40								
> 10–20	Chrysotile UICC							
> 5–10	+ + + +		11					
> 2.5–5		4		4				
> 0.5–2.5	8	15	14	11	17	7	5	3
> 20–40								
> 10–20	AAA glass crude fibre							
> 5–10	+ + + +							
> 2.5–5		1					3	7
> 0.5–2.5	< 1	< 1	1	2	2	5	13	65
> 20–40								
> 10–20	AAA glass separated "> 10 × 1 μm"							
> 5–10	+ + + +							
> 2.5–5		1			3			
> 0.5–2.5	8	3	6	17	18	10	23	12
> 20–40								
> 10–20	Aluminium oxide whiskers			4	4			
> 5–10	+ + + +		3	1	4	8		26
> 2.5–5		1	1	1	5	2	12	6
> 0.5–2.5	< 1	< 1	1	2	5	4	4	6

[a] +'s indicate extent of pleural fibrosis most commonly observed.

ball mill to produce partially or fully pulverised samples. The crude fibres were stripped by hand from hand-cobbed ore specimens, and bundles of fibres were retained as long as possible without contamination by extraneous mineral.

The fibrous glasses were obtained from both the Owens-Corning Fiberglas Corporation, Toledo, Ohio and the Johns-Manville Research and Engineering Center, Manville, New Jersey. We are particularly indebted to the latter institution for the size separation of fibrous glasses, which was carried out through a series of millings and the sedimentation of exceptionally fine-diameter glass fibres. All of the glasses were of the usual borosilicate type, whose mineral oxide contents have been previously recorded (Stanton & Wrench, 1972).

The non-fibrous aluminium oxide and aluminium oxide whiskers were commercial products obtained from the Artech Corporation, Falls Church, Virginia. These are single crystal fibres that are more than 99.5% pure Al_2O_3. The method of counting fibres has been described previously (Stanton & Wrench, 1972).

Samples of the materials, suspended in Formvar, were air-dried on glass slides and photographed at 1000× magnifications. From the photographs, 1000 consecutively counted particles were assigned to the 30 ranges of dimension indicated in Figure 1. Assuming that the particles in a given range were normally distributed around the mean size of that range, the total mass of all particles could be calculated; and it is the percentages of the total mass occupied by particles in a given range or size compartment that are presented in Tables 1–4.

In the tables, the first entry for each diameter and half of the diameters in the second entry in each row represent particles that are non-fibrous by optical standards. The plus figures below the designated specimen indicate the extent of pleural fibrosis most commonly observed in the rats in each experiment.

The rats are being observed for two years following application. A necropsy is performed on all dead or sick rats, and histologic sections are taken from the site of treatment and from any other abnormal lesion.

CONCLUSIONS

The data are arranged in four tables according to our estimates of mesothelioma incidence. In Table 1 are shown the five specimens which produced tumour incidences greater than 40%. These are the UICC standard reference samples of crocidolite and chrysotile A, two samples of very fine fibrous glass with diameters of 3 μm or less, and the aluminium oxide whiskers. All of these samples are composed almost entirely of fibres, and further have in common a predominance of fibres below 5 μm in diameter. The Al_2O_3 fibres are of particular interest because they are totally different from those of asbestos and glass, both in internal structure and in chemical composition; yet their size distribution is remarkably like that of UICC crocidolite. However, one-third of the fibres are slightly longer and thicker than are the crocidolite fibres and, since the density of Al_2O_3 is greater than asbestos, approximately one-sixth as many fibrous particles are present. The Al_2O_3 fibres are most durable and do not fragment into submicroscopic particles in the manner of asbestos. Nevertheless, electron microscope study reveals an abundance of submicroscopic fibrils of less than 0.1 μm in diameter with lengths comparable to those of fibres in the lowest optical range.

Tables 2 and 3 list the six samples of asbestos and glass that fall in the middle ground of carcinogenicity. These groups show several outstanding differences in fibre distribution from those shown in Table 1; both extremes in the dimensional range of fibres are represented. For example, the two crocidolite samples show similar decreases in carcinogenicity; however, one is composed almost entirely of long, large-diameter fibre bundles (Table 2); while the other (Table 3) has nearly half its mass reduced to fibres that are at the very lowest optical range of diameter and length and the rest reduced to particles too small to be recognised as fibres by optical standards. This latter fraction is of particular importance, since it consists of particles that are aggregates of submicroscopic fibrils with diameters below 0.2 μm and with lengths of 5 to 10 μm. Since these very short fibrils of submicroscopic size account for the bulk of this material and occur in far greater numbers than in the non-pulverised crocidolite, it follows that their role in carcinogenesis is probably negligible (Stanton & Wrench, 1972).

The distributional array of fibres in the three glass samples of these groups indicates the second dimensional parameter of carcinogencity. Here it is apparent that carcinogenicity decreases as more fibres exceed 2.5 μm in diameter and as the fibre length decreases to less than 10 μm. Again, the submicro-

Table 2. Moderate incidence groups (30–20% mesotheliomas). Percentage of mass occupied by fibres in each range of size

Diameter μm	Length μm							
	> 0.5–2.5	> 2.5–5	> 5–10	> 10–20	> 20–40	> 40–80	> 80–160	> 160–320
> 20–40								42
> 10–20	Crocidolite crude fibre							22
> 5–10	+ +[a]						1	10
> 2.5–5							2	12
0.5–2.5	< 1	< 1	< 1	1	2	2	3	2
> 20–40								
> 10–20	Chrysotile long fibre							20
> 5–10	+ + +							16
> 2.5–5		2					10	12
> 0.5–2.5	4	2	1	4	2	7	6	14
> 20–40								
> 10–20	AAA glass sheared fibre			17				
> 5–10	+ + + +		4					
> 2.5–5		4	2	2	11	9	8	
> 0.5–2.5	7	2	4	9	10	2	1	8
> 20–40								
> 10–20	AAA glass separated "> 10 × 3 μm"			2	4			
> 5–10	+ + +		1	1	15	10		
> 2.5–5		3	2	9	22	9	8	10
> 0.5–2.5	< 1	< 1	< 1	1	2	< 1	< 1	< 1
> 20–40								
> 10–20								
> 5–10								
> 2.5–5								
> 0.5–2.5								

[a] +'s indicate extent of pleural fibrosis most commonly observed.

scopic fractions of these samples contain an abundance of fibres below 0.2 μm in diameter, with distributions of length similar to those of fibres present in the lowest optical range.

Finally, Table 4 lists those materials that have yielded less than 5% incidence of mesotheliomas. No tumours have developed in rats exposed to either crocidolite or chrysotile pulverised to the extent of reducing all of the optically visible particles to non-fibrous form. Since these excessively milled materials contain all of the elemental components of asbestos and are contaminated by metallic erosion of the mill far more than are the other samples, it follows that neither of these factors is a likely source of asbestos carcinogenicity and that structural integrity of the fibre is essential. The two glass samples in this group again represent both extremes in structure, namely either fibres of great length with diameters greater than 10 μm or particles that are short, thick and essentially non-fibrous by optical standards. In the non-fibrous Al_2O_3 experiment, a single tumour has been observed among 30 rats. This tumour is of doubtful significance, but it may reflect the low background incidence of less than 5% induced by partial fragmentation of the vehicle.

In summary, the experiments may be compared among single types of material. The results from the seven samples of asbestos indicate that none of the three extremes in fibre distribution yield as high an incidence of mesotheliomas as do the more evenly distributed UICC standard reference samples. Either progressive pulverisation to non-fibrous form or preservation of the test sample in large bundles of fibres clearly reduces carcinogenicity. In comparing the seven glass samples it is apparent that samples composed over 90% by weight of fibres with diameters of 2.5 μm or less are the most carcinogenic; and that as length is reduced in these small fibres carcinogenicity is also reduced. Finally, the contrasting results obtained using fibrous and non-fibrous forms of Al_2O_3 re-emphasise the importance of structure in carcinogenicity. These exceptionally pure, inert fibres, composed of materials foreign to asbestos and glass, seem to carry the same carcinogenic hazard for the pleura as do those materials.

It would seem therefore that carcinogenicity is in

Table 3. Low incidence groups (20–10% mesotheliomas). Percentage of mass occupied by fibres in each range of size

Diameter μm	Length μm							
	> 0.5–2.5	> 2.5–5	> 5–10	> 10–20	> 20–40	> 40–80	> 80–160	> 160–320
> 20–40								
> 10–20	Crocidolite partly pulverised							
> 5–10	+ + +[a]							
> 2.5–5								
> 0.5–2.5	54	20	17	9				
> 20–40								
> 10–20								
> 5–10								
> 2.5–5								
> 0.5–2.5								
> 20–40								
> 10–20	AAA glass partly pulverised							
> 5–10	+ + +		< 1	2		16	32	
> 2.5–5		4	4	2	8	6	4	8
> 0.5–2.5	1	2	3	2	2	1	1	3
> 20–40								
> 10–20								
> 5–10								
> 2.5–5								
> 0.5–2.5								
> 20–40								
> 10–20								
> 5–10								
> 2.5–5								
> 0.5–2.5								

[a] +'s indicate extent of pleural fibrosis most commonly observed.

Table 4. Negligible incidence groups (< 5% mesotheliomas). Percentage of mass occupied by fibres in each range of size

Diameter μm	Length μm							
	> 0.5–2.5	> 2.5–5	> 5–10	> 10–20	> 20–40	> 40–80	> 80–160	> 160–320
> 20–40								
> 10–20	Crocidolite fully pulverised			20				
> 5–10	+[a]		14					
> 2.5–5		26						
> 0.5–2.5	40	< 1						
> 20–40					30			
> 10–20	Chrysotile fully pulverised			28				
> 5–10	+		25	5				
> 2.5–5		5	2	< 1				
> 0.5–2.5	3	< 1	< 1	< 1				
> 20–40								47
> 10–20	Commercial glass whole fibre							24
> 5–10	+							21
> 2.5–5						< 1	< 1	7
> 0.5–2.5						< 1	< 1	1
> 20–40								
> 10–20	AAA glass separated " < 5 × 3 μm"				29			
> 5–10	+		6	2	4			
> 2.5–5		5	9	20	14	2		
> 0.5–2.5	3	2	3	1	< 1			
> 20–40					28			
> 10–20	Aluminium oxide non-fibrous			31	19			
> 5–10	+		10	4				
> 2.5–5		5	1					
> 0.5–2.5	2	< 1						

[a] +'s indicate extent of pleural fibrosis most commonly observed.

some way related to the presence of durable particles of fibrous configuration that are particularly long at or perhaps below the smallest diameter which can be recognised optically; and that the carcinogenicity of these fibres has little relation to their chemical composition or to their potential contaminants.

SUMMARY

Various structural forms of asbestos, fibrous glass and aluminium oxide have been tested for carcinogenicity on the pleura of rats. Results from all three materials indicate that carcinogenicity is related primarily to fibrous structure rather than to physicochemical properties. A comparison of the dimensional distribution of fibres in those samples of asbestos and glass producing high and low tumour incidence indicate that carcinogenicity may be related to fibres below 2.5 μm in diameter and between 10 to 80 μm in length.

ACKNOWLEDGMENTS

We wish to thank Dr Vernon Timbrell for advice in the analysis of the data; Mrs Constance Wrench and Miss Eliza Miller for their diligence in monitoring the experiments; and the staffs of the Owens-Corning Fiberglas Corporation, the Johns-Manville Research and Engineering Center, and the MRC Pneumoconiosis Unit, Penarth, UK, for generously providing many of the materials used.

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Physical factors as etiological mechanisms

V. TIMBRELL [1]

Prior to the 1964 New York meeting little attention had been given to the possible importance of physical factors in the etiology of lung diseases associated with exposure to asbestos dust. Animal experiments had indicated that the fibrogenicity of asbestos particles was probably related to fibre length (Vorwald *et al.*, 1951; Wagner, 1963; Holt *et al.*, 1964); but despite the fact that the relevant occupational exposures were by inhalation, a study described at the 1964 meeting (Timbrell, 1965) on the aerodynamic properties of fibres seems to have been the first attempt to investigate these aspects. Subsequent inoculation experiments (Burger & Engelbrecht, 1970) have re-emphasised the importance of fibre length in relation to fibrosis; while very recent intrapleural inoculation studies (Stanton & Wrench, 1972; Wagner *et al.*, 1972 [2]) have indicated a relationship between fibre size and the development of mesotheliomas. In the light of this evidence it is not surprising that studies of the physical characteristics of fibres in recent years have been profitable and now offer plausible explanations for some of the biological observations.

Studies of possible etiological factors are assisted by two features of asbestos minerals and of the associated lung diseases. First, asbestos minerals are crystals and as such exhibit certain constant physical properties: some of these features are illustrated in Figure 1. It is no small advantage in analysis that the particles are consistently of fibrous shape, thus effecting a major reduction in the number of possible particle shapes compared to, for instance, coal; and that a few samples from an asbestos geological entity appear to be sufficient to give a good indication of the particle characteristics of the whole deposit, probably back to the time it was first worked. Secondly, the epidemiological, pathological and experimental evidence presents a series of paradoxes which are useful in testing ideas concerning the etiology. As an example, the major difference in the incidence of mesotheliomas in north-western Cape Province compared with that in the Transvaal suggests a need to search for some factor (or factors) in the asbestos minerals that consistently have very different magnitudes in the two areas. In this instance it seems likely that such a factor is not chemical because, although there are variations in mineral content of the fibres, these differences do not appear sufficient to provide an explanation. In contrast, some of the physical properties differ so markedly that they impress as possible etiological factors.

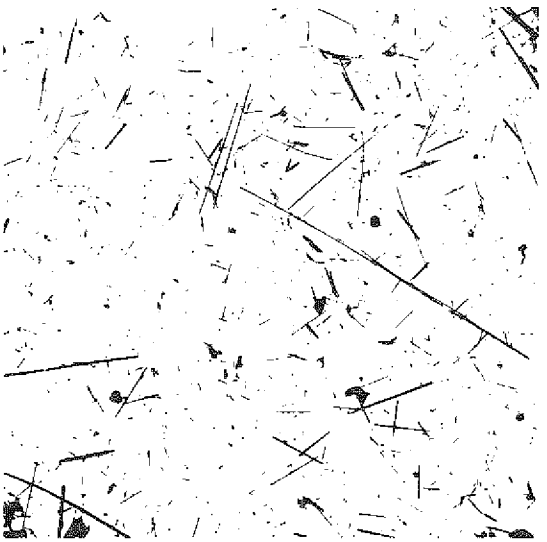
FIBRE IN LUNGS

The aerodynamic study presented at the New York meeting provided explanations for some of the features of asbestos fibres detected in lung sections. The deposition of particles in lung airways is related to the aerodynamic diameter of the particles as measured by their free-falling speed. Most inhaled particles with falling speeds greater than that of a unit density sphere 10 μm in diameter are deposited in the upper bronchial tree by sedimentation or by impaction. So the demonstration that an asbestos fibre about 3 μm in diameter has the same falling speed as that of a 10 μm sphere of unit density indicates that fibres of this diameter are the thickest that are likely to penetrate to the alveolar regions and to be eventually seen in lung sections. This conclusion is in good agreement with measurements made on fibres in lungs of city dwellers (Gross *et al.*, 1971) and with measurements made on fibres in

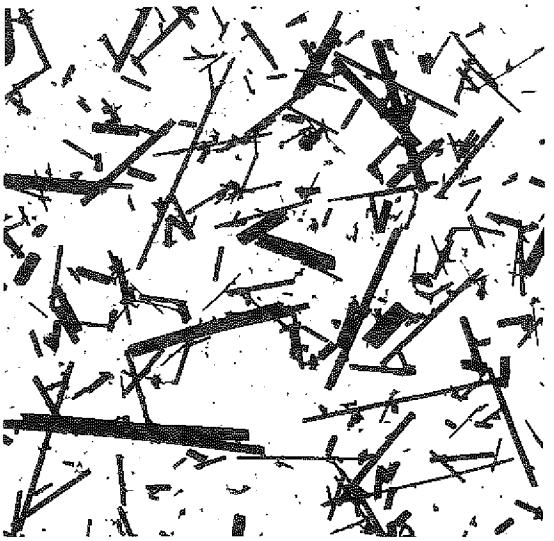
[1] MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, UK.

[2] Unpublished data.

CROCIDOLITE



AMOSITE



ANTHOPHYLLITE

CHRYSOTILE

10 μm

lungs of rats exposed to airborne particles of amphibole asbestos (Timbrell *et al.*, 1970). It may be noted here that rat lungs have often provided data on particle deposition, retention and clearance which are comparable with those obtained from human lungs.

Calculations made in this aerodynamic study indicated that interception is likely to prevent fibres longer than 200 μm from penetrating the nasal passages. However, interception is not effective in depositing fibres, not even long ones, in the trachea and other wide airways of the upper bronchial tree. The conclusion that long fibres which are thin enough not to be deposited earlier by sedimentation and impaction can reach the alveolar regions explains satisfactorily the numerous long asbestos fibres detected in lung sections.

Further calculations indicated that long fibres which reach the respiratory bronchioles are efficiently deposited in these fine tubes, and that bifurcations are the preferred deposition sites. It has been suggested that long fibres are transported from these pulmonary regions less efficiently than are short fibres because they find anchorage in these complicated anatomical structures more readily than do short fibres (Timbrell & Skidmore, 1968), and that this may explain why fibrosis tends to be associated first with the respiratory bronchioles (Wagner, 1963) and with long fibres. These conclusions are in good agreement with the detection of accumulations of long fibres in small airways, especially at bifurcations; but the possibility of transportation of fibres to these regions from other deposition sites cannot be eliminated. Current studies on rats exposed by inhalation to radioactive crocidolite fibres (Morgan, 1972 [1]) are likely to produce much needed data on the pulmonary deposition pattern of fibres and their subsequent clearance.

Little information is available on the important question of the relative efficiency of pulmonary penetration of fibres of amphibole asbestos and chrysotile. In an experiment reported by Wagner (1972), in which rats were exposed to dust clouds of the UICC samples at equal concentrations, six times as much of each of the amphibole samples was still retained after several months compared to the amount of chrysotile. Timbrell (1970; 1972a) has suggested that this may be partly due to the characteristic rectilinear shape of amphibole fibres, compared to the curly morphology of chrysotile fibres, which could cause amphibole fibres to penetrate to the deeper parts of the lung more efficiently than do chrysotile fibres. The results of studies in lung casts and narrow tubes, which showed a relative penetration of amphibole and chrysotile fibres similar to that obtained in the animal experiment, are offered in support of this explanation. It is difficult to estimate what influence the tendency of chrysotile fibres to break down chemically and physically after prolonged residence in the lung (Langer *et al.*, 1971) may have had on the result of the animal experiment. The contribution may not be substantial since the asbestos content of the lungs was estimated from the silica present, and it appears that whereas magnesium is rapidly leached from a chrysotile fibre *in vivo* (Morgan & Holmes, 1970) a silicate skeleton remains.

There is a lack of information on whether asbestos fibres can reach the pleura during inhalation or whether they migrate to this region. This question is of particular interest in a consideration of the development of mesotheliomas. In an experiment to compare the effects of long (5–200 μm) fibres and small particles of the UICC crocidolite sample in monkeys and baboons, Rendall (1972 [2]) found that small particles are not fibrogenic but that they can reach the pleura in macrophages. Results available from a current electron microscope study on the lungs of rats exposed to dust clouds of the UICC samples at equal concentrations suggest that asbestos fibres can reach the alveoli near the pleura during

[1] Personal communication.

[2] Personal communication.

Fig. 1. Electron micrographs of crocidolite (north-western Cape Province), amosite (Transvaal), anthophyllite (Finland), chrysotile (Canada) at the same magnification (×1700). Features to note are:

(i) the rectilinear shape of the amphibole fibres compared with the curved and twisted morphology of chrysotile fibres;
(ii) the order of the diameters of the amphibole fibres,

crocidolite < amosite < anthophyllite,

which numerous e.m. examinations suggest is a characteristic of these fibres in the three geographical areas;
(iii) the longitudinal fragmentation of the chrysotile fibres and the small diameter (about 0.03 μm) of the ultimate fibrils,

inhalation. In animals killed immediately after a short exposure to crocidolite, small numbers of these fibres have been detected near the visceral pleura; some fibres have been detected in the pleura itself, although this could be an artefact.

The numbers of amosite fibres observed near the pleura have been lower, as might be expected from the greater diameter and higher falling speed of this type of fibre.

The numbers of anthophyllite fibres detected near the pleura have been still lower, as might be expected from the still greater thickness of this fibre: the fact that anthophyllite fibres are capable at all of reaching the pleura during inhalation may be due to their lath-like shape (Timbrell *et al.*, 1970) which gives them lower falling speeds and thus greater penetration than they would have if they were of circular cross-section.

Chrysotile fibres have very seldom been observed near the pleura in this study. This suggests support for the conclusion, derived from considerations of the morphology and the aerodynamic behaviour of chrysotile fibres, that this type of asbestos does not penetrate efficiently to the deeper parts of the lung. But the fact that, in a current inhalation experiment, UICC Canadian chrysotile has produced mesotheliomas, suggests that the present examination techniques are not adequate to detect and quantify asbestos fibres in tissue, particularly very fine chrysotile fibres.

In a recent study Pooley (1972) used the electron microscope to examine chrysotile fibres in sections of 300 human lungs. The majority of the chrysotile particles were grouped together in large clusters of strands (bundles of single chrysotile fibres), which were often detected at the bifurcation of small airways. Very short individual fibres were also found scattered throughout the lung sections. This distribution of short chrysotile fibres resembles that of amosite fibres in the lungs of rats used in an inhalation experiment (Timbrell & Skidmore, 1968). Considerations of fibre morphology suggest an explanation for these results: short and long chrysotile fibres characteristically have the same radii of curvature, so that short chrysotile fibres, being short arcs, are virtually straight and behave aerodynamically like amphibole fibres. This indicates that chrysotile and amphibole fibres will exhibit different aerodynamic behaviour if they are long, but similar behaviour if they are short.

OCCUPATIONAL EXPOSURES

Although a substantial amount of dust sampling has been carried out to monitor occupational exposures to asbestos, very little sampling has been done to provide basic information on the nature of the airborne particles. In particular, there has been a lack of investigations designed to compare the nature of dust clouds in amphibole and in chrysotile exposures, and to compare these with experimental clouds. Major differences must be expected.

Whereas the animal experiment reported by Wagner (1972), using the UICC samples, indicated a 6:1 retention of amphiboles compared to chrysotile, substantially larger ratios may be expected to occur in occupational exposures. Both the amphibole and chrysotile UICC samples were specially prepared so as to be rich in fibres of respirable size. Measured by Hexhlet-type instruments, which take account of particle falling speed only, the proportions by weight of respirable particles are high (about 70%) and approximately equal in all the UICC samples. Furthermore, the samples contain only a small proportion by number of long fibres (up to 200 μm); and only these fibres, in conjunction with the difference in fibre morphology, could contribute to a difference in particle deposition patterns between amphibole and chrysotile in this animal experiment. In contrast, the industrial preparation of chrysotile is controlled to avoid excessive opening of the fibres (Sinclair, 1959) and the production of a high proportion of the fine fibrils; whereas milling experiments (Timbrell & Rendall, 1971/72) have shown that it is not possible to mill the amphibole asbestos types without producing extremely fine fibres: some crocidolites, for example that from north-western Cape Province, are particularly rich in fine fibre. The dense clouds of dust and the layers of settled dust often seen in chrysotile mining areas consist largely of relatively thick strands and big flocks which settle rapidly and are available for inhalation only for limited periods: some of these particles are of such large aerodynamic size that many types of dust sampling instruments do not even aspirate them. Rendall & Timbrell using Hexhlet-type instruments observed some low values (5%) for the proportion of respirable particles in air samples collected in some South African chrysotile mills, while Gibbs (1971) has reported low values (range 14–25%) in Canadian mills; but there is a great need for more data on chrysotile and amphibole

milling and other processes, for comparison purposes. A study with this objective is in progress.

These considerations suggest that even in occupational exposures to chrysotile enough fibre may reach the bronchial regions of the lung to produce fibrosis and carcinoma, but that the amphibole asbestos types are more likely to produce these diseases because of higher effective exposures. Although the possibility of some chrysotile fibres reaching the pleura clearly cannot be dismissed, especially the short fibres detected by Pooley, this type of asbestos appears to be the least likely to be associated with pleural mesotheliomas from occupational exposures if penetration of fibre to the deeper alveoli adjacent to the pleura is an important factor in the production of these tumours.

There is also a need for information on the nature and concentration of airborne particles at various stages in the preparation of asbestos. It has been pointed out by Timbrell & Holmes (1970) that the application of fiberising processes must progressively increase the number of fibres and must make them more easily respirable because of the decrease in their diameters. This suggests that there may be major differences in exposures at different points in the industry, even when only one type of fibre is involved. That such differences do occur is supported by evidence that health risks have been high in the asbestos textile trade (Merewether & Price, 1930; Doll, 1955; Enterline & Kendrick, 1967; Mancuso & El-Attar, 1973; Newhouse, 1970), where the fibres are at their finest, shortest and cleanest.

PLEURAL MESOTHELIOMAS IN THE NORTH-WESTERN CAPE AND THE TRANSVAAL

Probably the greatest paradox presented by asbestos diseases is the clear association between the development of mesotheliomas and exposure to crocidolite dust in the north-western Cape (Wagner *et al.*, 1960; Oettlé, 1964), compared with the very rare occurrence of these tumours in the Transvaal where both crocidolite and the closely related amosite are mined (Oettlé, 1964; Harington *et al.*, 1971). This marked difference in mesothelioma risk is not satisfactorily explained by differences in the commercial production of fibre in the two areas (Keep, 1961; Harington *et al.*, 1971), nor by mineralogical factors, nor by the occurrence of asbestosis (Sluis-Cremer, 1965).

Cralley (1971) has suggested that the difference is due to the electromotive influence of the excess elemental manganese in the respirable dust of north-western crocidolite over that in the respirable dust of Transvaal crocidolite, whose parent rock is softer and requires less harsh milling treatment to free the fibre. The estimated three-fold excess of manganese does not, however, seem sufficient to explain the sharp disease pattern; but it is not possible to dismiss metals as the ultimate carcinogen, with the fibres acting as carriers. Suggestions that differences in oil content of the fibres in the two areas may be responsible are not supported by intrapleural inoculation experiments in which oil-extracted UICC crocidolite from a north-western Cape mine produced a very similar mesothelioma rate to that with untreated fibre (Wagner *et al.* 1972 [1]).

A recent suggestion is that differences in particle size may be important (Timbrell *et al.*, 1971). Electron microscope studies on fibres prepared by grinding rock specimens from the two areas showed a consistent difference in fibre size: Transvaal fibres, both crocidolite and amosite, were on average three times the diameter, three times the length, and hence 27 times the volume or mass of north-western Cape fibres. Very recent studies on air samples have confirmed these differences which, it has been suggested, indicate for the Transvaal compared to the north-western Cape less liberation of respirable fibres; higher fibre settling rates and shorter times available for inhalation; comparable deposition rates in the upper bronchial tree, but less efficient penetration to the deeper parts of the lung. The last comparison may explain the apparent lack of association between mesotheliomas and asbestosis in the two areas.

Electron microscope examinations of samples of Australian crocidolite have shown that the fibres are even smaller in diameter than those of north-western Cape crocidolite (Timbrell *et al.*, 1970). This may be related to reports from Australia (Wagner *et al.*, 1971) that mesotheliomas have occurred following exposure to crocidolite in the mining areas, and to the fact that mesotheliomas have been observed in workers who were exposed to Australian crocidolite when packing respirators during World War II (Jones, 1968 [2]).

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[1] Unpublished data.
[2] Personal communication.

INTRAPLEURAL INOCULATION EXPERIMENTS

The development of mesotheliomas in rats following intrapleural implantation or inoculation of fibrous materials has been studied in two recent experiments by Stanton & Wrench (1972) and by Wagner *et al.*, (1972 [1]). In these experiments chrysotile exposure, which in general presents a smaller mesothelioma hazard for man than does exposure to crocidolite (Wagner *et al.*, 1971), has produced mesotheliomas as readily as the latter. A probable explanation for this paradox is that such inoculation experiments do not take account of the natural factors which operate during inhalation or, consequently, of the important differences in the aerodynamic behaviour of fibres of the two main asbestos groups.

In such inoculation experiments, sizing of the particles by electron microscopy is necessary, since some of the materials may contain a high proportion of particles that are not visible in the light microscope. The data available on the physical and chemical characteristics of these materials are so complex that some indication of the important factors is essential for successful interpretation of biological results. Suggestions of which physical factors may be involved have been provided by recent studies on fibre-cell preparations. In investigations based on the working hypothesis that asbestos cancers may be related to the implantation of fibres in cells (which does not immediately kill the cells), the preferred orientations that fibres of amphibole asbestos types exhibit in a magnetic field (Timbrell, 1972b) have been used to apply the fibres end-on to the membranes to impale the cells. These studies have indicated that if fibres are thinner than a threshold diameter they may be thus implanted without destroying the morphology of the cells: the threshold diameter and its range remain to be determined, a tentative value being set at 0.5 μm. Similar experiments have emphasised that fibres are of an ideal shape to transmit force and motion over considerable distances in cell agglomerations, and that these features are functions of fibre length; also, that whereas fibres which are short compared with cell diameters are able to move comparatively freely, longer fibres readily find anchorage, giving rise to the possibility of relative motion between fibre ends and cells and of irritation and penetration of membranes. These observations suggest that if the carcinogenesis associated with asbestos is related to such mechanical action there may be a threshold length, of the order of the size of the cell diameters, that the fibres must exceed in order to produce this biological response.

A further factor that must be taken into account in examining the results of intrapleural inoculation experiments is the tendency of chrysotile fibres *in vivo* to fragment longitudinally into fine fibrils (Suzuki & Churg, 1969); thus the degree of fragmentation and hence the number of fibres produced can be expected to depend on variations in physical and physiological conditions (Timbrell, 1972a). Fibres of the amphibole types of asbestos, on the other hand, appear to retain their morphology and characteristic diameter distributions (Timbrell *et al.*, 1970).

Although a direct relationship between these physical factors and the development of mesotheliomas has not been demonstrated, they are the relevant factors in the Stanton & Wrench (1972) and the Wagner *et al.* (1972 [2]) experiments, if it can be considered that consistency in the interpretation of the biological observations made in these two studies is a satisfactory test. In examining the results, fibre diameter is once more found to be a central factor, largely because the number of fibres in a given quantity of material placed in the pleural cavity (in each study a standard weight of each material was inoculated or implanted) is determined more by fibre diameter (inversely proportional to diameter^2) than by fibre length.

When the non-chrysotile materials (samples of crocidolite, amosite, chrysotile, brucite, synthetic aluminium silicate fibre and aluminium oxide particles) used in the Wagner *et al.* (1972 [2]) experiment are classified according to fibre diameter, the order obtained is in good agreement with the order in terms of the number of mesotheliomas produced: the finer the fibre the greater its carcinogenicity. The *in vivo* dispersion of chrysotile materials and their fibre diameters are difficult to quantify, but their position in the classification by carcinogenicity is understandable from their fragmentation and agglomeration characteristics. In the Stanton & Wrench (1972) experiment a total of 17 samples, including samples of several types of asbestos as well as of fibrous glass and metallic nickel fragments, were used. When the results reported in that study are

[1] Unpublished data.

[2] Unpublished data.

examined using the light microscope data provided by those authors for the size of the coarser materials and the electron microscope data collected by Timbrell for the size of the amphibole asbestos types, there is again good agreement between the order of the materials in terms of fibre diameter and their classification according to the number of mesotheliomas produced. The formation of mesotheliomas by fine glass fibres in this study, in contrast to the absence of these tumours in an earlier Wagner [1] experiment using coarser glass fibre, is consistent with the concept of a threshold fibre diameter. Furthermore, the observation made in the Stanton & Wrench experiment that the number of mesotheliomas produced decreased with degree of pulverisation of the materials is consistent with the concept of a threshold fibre length. Further experiments are needed using additional types of fibrous materials, and preferably employing fibres in narrow ranges of diameter and length.

POSSIBLE FACTORS IN CARCINOGENESIS

The fact that the fibrous form of a range of materials (including several types of asbestos, brucite, synthetic aluminium silicate and glass) produces mesotheliomas suggests that particle morphology may be an essential factor. The fact that fibrous materials differing greatly in chemical composition produce mesotheliomas suggests that the initiation of a tumour is unlikely to be due to special characteristics of the materials, for example, a certain type of molecular structure. It may be significant that fibres appear to be more carcinogenic the closer their diameters approach the thickness of cell membranes and the size of cell organelles.

CONCLUSIONS

Extracellular processes and the physical features of fibres thus appear to constitute a major part of the etiology of the lung diseases associated with exposure to asbestos dust, including the cancers. The biological responses which different types of asbestos fibres produce when inhaled seem to be governed largely by the aerodynamic properties of the fibres. This apparent deep involvement of physical factors suggests that enough basic information is already available to indicate the engineering measures needed to minimise the health risks, and that knowledge of the cellular processes may contribute little to the achievement of this objective. However, further investigations of asbestos cancers may provide valuable clues for the elucidation of mechanisms of carcinogenesis.

[1] Unpublished data.

SUMMARY

Studies in recent years have indicated that physical factors are deeply involved in the etiology of lung diseases associated with exposure to asbestos dust, and these studies now offer explanations for some of the biological observations. The physical characteristics of the fibres which form the basis of these suggested explanations are fibre diameter, fibre length and fibre morphology, the central parameter being fibre diameter. In this paper, physical factors are discussed as possible etiological mechanisms, and the apparent marked influence of fibre size in two very recent intrapleural inoculation experiments is described.

ACKNOWLEDGMENTS

I am grateful to my colleagues Mr G. Berry, Dr J. C. Gilson, Dr F. D. Pooley and Dr J. C. Wagner for many valuable discussions and to Dr J. S. P. Jones, Mr A. Morgan, Mr R. E. G. Rendall and Dr M. F. Stanton for access to unpublished material.

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Chemical factors (including trace elements) as etiological mechanisms

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"Asbestos" is the generic name given to a class of fibrous mineral silicates which vary considerably in physical and chemical composition (Hodgson, 1965; Cape Asbestos Group, 1972). The demonstration of differences in the fibrogenicity and carcinogenicity among different types of asbestos, either in epidemiological or experimental studies, would greatly clarify the mode of action of each type of fibre.

ASBESTOS AS A FIBROGENIC AGENT

If the fibrogenic action of silica appears a little more straight-forward than it did ten years ago (see Harington, 1969), that of asbestos continues to baffle. This is partly because there are many forms of asbestos, and partly because asbestos, using the term in the general sense, is carcinogenic as well as fibrogenic. Thus, it may be wise, for experimental purposes at least, to distinguish between these two modes of action. It is possible for example that all or most forms of asbestos so far studied are, under the "correct" conditions, fibrogenic and therefore cytotoxic; whereas it is already known that not all forms of asbestos are carcinogenic, or that some are much less so than others.

Comparison of the behaviour of asbestos with that of silica after phagocytosis has so far been illuminating; but such approaches are by no means straightforward. The experiments in which macrophages have been exposed to different forms of asbestos have shown that on a short-term basis, chrysotile seems much more cytotoxic than others (Koshi *et al.*, 1968; Bey & Harington, 1971; Miller & Harington, 1972; Harington, 1972). Indeed, on the basis of these findings, crocidolite and amosite could be regarded as being biologically "inert". This is borne out by Koshi *et al.* (1968), who found good correlations between the toxicity of silica and chrysotile asbestos and their ability to release acid phosphatase, a "marker" lysosomal enzyme, from macrophages in culture. In their work, crocidolite and amosite appeared to be non-toxic.

However, because in man and animals most forms of asbestos, including chrysotile, crocidolite, amosite, anthophyllite and tremolite, are actively fibrogenic, it seems correct to infer that the technique of macrophage culture as it is at present used in work on the cytotoxicity of asbestos may not be adequate as a means of explaining how different forms of asbestos fibres affect the cell, or that asbestos may not need to be cytotoxic to be fibrogenic. The technique of cell culture seems to need detailed reappraisal so that longer exposure of macrophages to various forms of asbestos can be obtained. Some forms, for example, crocidolite and amosite, may have to be present for a long time before they damage macrophages (Harington, 1972). In addition, recent experiments using haemolysis as an indication of cytotoxicity suggest that certain factors in serum, possibly the complement system, may accelerate the action of apparently inert forms of asbestos (Harington *et al.*, 1971b).

As far as phagocytosis is concerned, it seems clear that all forms of asbestos so far studied are taken up into phagosomes in much the same way as is silica, although asbestos often lies free in the cytoplasm. Several studies using electron microscopy have shown clearly that this is the case (Dourmashkin & Dougherty, 1961; Richter, 1961; Davis, 1963, 1973;

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Allison, 1967; Harington *et al.*, 1973; Suzuki *et al.*, 1973). However, there is no convincing evidence that asbestos damages the membranes of phagosomes in the way that silica does, although recent work by Smith & Davis (1972) has shown the presence of acid phosphatase—indicative of lysosomal activity—in phagosomes of cells given asbestos. Further support for such lysosomal effects may be found in recent work by Miller & Harington (1972). There is also the possibility that mechanical damage to the phagosomal membrane may occur after contact with asbestos (Davis, 1973).

The haemolytic propensity of different forms of asbestos is better understood. Chrysotile is as strong a haemolytic agent as is silica powder (Macnab & Harington, 1967; Secchi & Rezzonico, 1968; Koshi *et al.*, 1968; Schnitzer & Pundsack, 1970; Harington *et al.*, 1971c). The principal reactant on the chrysotile surface is probably magnesium in its ionic form, and not hydrogen as is the case with silica (Nash *et al.*, 1966). This view is supported by the finding that the chelating agent, disodium ethylenediamine tetraacetic acid (EDTA), and phosphate ions effectively prevent lysis of red blood cells by chrysotile, whereas polyvinylpyridine-N-oxide (PVPNO), which protects red cells against the lytic action of silica, is almost ineffective (Macnab & Harington, 1967; Harington *et al.*, 1971c).

Furthermore, a correlation is to be found between the haemolytic activity of different forms of asbestos and the magnesium:silicon ratio of each (Harington *et al.*, 1973); this may possibly apply to the cytotoxic action of asbestos on macrophages. The action of magnesium is not as a trace metal but that of a metal bound firmly to the lattice of the fibre itself, from where, in an ionic form, it exerts a marked catalytic or complexing effect. Finally, the weak haemolytic activity of several forms of asbestos, such as crocidolite and amosite, appears to be increased markedly when fresh serum (possibly the complement system) is present (Harington *et al.*, 1971b). Experiments in progress suggest that these principles might also apply to the weak cytotoxic action of certain forms of asbestos on the macrophage (Miller & Harington, 1972).

To sum up, it would seem that studies of the cytotoxicity of asbestos should take into account the length of time over which macrophages are exposed to fibres, the type of macrophage (and hence the type of phagosomal membrane) to which the different forms of asbestos are exposed, the type of dust used and the conditions of cell culture (see Harington, 1972).

ASBESTOS AS A CARCINOGENIC AGENT

Epidemiological considerations

Two types of malignancy in man are associated with exposure to asbestos: carcinoma of the lung, and mesothelioma of the pleura and peritoneum. The epidemiological features of these cancers have been dealt with by several authors (O'Donnell & Mann, 1957; Mancuso & Coulter, 1963; Oettlé, 1964; Buchanan, 1965; Selikoff *et al.*, 1965; Mancuso & El-Attar, 1973; Selikoff *et al.*, 1973). Attempts have been made to explain the apparently moderate effect of certain types of fibre, for example, amosite (Enticknap & Smither, 1964) as compared to that of crocidolite (Wagner *et al.*, 1960), especially as this applies to the South African asbestos fields (Sluis-Cremer, 1965; Harington & Roe, 1965; Harington, 1967a; Harington *et al.*, 1971a). These differences are not due to the commercial production of these two fibres (Keep, 1961; Stander & le Grange, 1963; Harington *et al.*, 1971a) or to other factors studied (Sluis-Cremer, 1965), although fibre diameter may be involved (Timbrell *et al.*, 1971).

Chrysotile is implicated in the development of mesothelioma (Enticknap & Smither, 1964; Elwood & Cochrane, 1964); and in the United States this asbestos seems to be actively involved as an etiological agent (Selikoff *et al.*, 1965; Mancuso & El-Attar, 1973).

Experimental considerations

Results of animal experimentation so far available suggest that crocidolite and chrysotile are more active in inducing mesotheliomas than is amosite (Wagner, 1962). In more recent work, Wagner & Berry (1969 [1]) and Wagner *et al.* (1972 [1]) found crocidolite and chrysotile to be carcinogenic to rats. A high proportion of most of the treated groups developed mesotheliomas. Brucite, the magnesium hydroxide outer layer of chrysotile, was also carcinogenic.

Possible modes of action of asbestos as a carcinogen

The following possible mechanisms of carcinogenesis have been considered in the past, or are under

[1] Unpublished data.

consideration at present: (1) that carcinogenesis might be due to certain carcinogenic metals or metal-complexes in asbestos, or to contaminating trace metals on the fibre; (2) that it might be due entirely, or in part, to organic materials on or in the fibre; (3) that it might be an "Oppenheimer effect" (Roe, 1966); and (4) that it might be associated with some physical attribute of the fibre, for instance, diameter or fineness.

(1) *Metal carcinogenesis and asbestos*

Asbestos as a metal complex

The possibility that metals may be involved in asbestos carcinogenesis seems to merit consideration (Harington, 1965; Harington & Roe, 1965). Many metals have been shown to be capable of inducing cancer (Roe & Lancaster, 1964; Furst & Haro, 1969); among the more notable are arsenic, chromium, nickel, cobalt, cadmium, lead and beryllium. In addition, several others, though not apparently carcinogenic in the simple ionic state, become so when combined in certain macromolecular complexes. The best-known example of this is iron, which as ferrous sulphate or gluconate shows no activity, but which in the forms of iron-dextran, iron-dextrin, saccharated iron oxide or ferrous glutamate is carcinogenic (Haddow *et al.*, 1961; Richmond, 1959; Haddow & Horning, 1960). Aluminium is another example, since aluminium dextran is a carcinogen (Haddow *et al.*, 1961). Against this background it is interesting to consider the metal contents of certain types of asbestos. The amphibole asbestos types, crocidolite and amosite, are complex silicates containing iron, magnesium, calcium and sodium in varying proportions. Chrysotile is a magnesium silicate with relatively small amounts of iron but with much magnesium (Hodgson, 1965).

The effect of introducing excess iron in a relatively stable state into tissues and cells has been discussed in detail elsewhere (Harington & Roe, 1965). The action of large amounts of magnesium (free or in a metal complex) is not known (Furst & Haro, 1969), although the metal in asbestos is known to be membrane-active (Macnab & Harington, 1967; Harington *et al.*, 1971c).

Trace metals in asbestos

The aspect so far discussed concerns the part played by metals as integral features of the molecular structure of asbestos. Spectrochemical trace analyses of different forms of asbestos carried out by Harington (1965), Cralley *et al.* (1967), Timbrell (1970) and Holmes *et al.* (1971), however, have shown the presence of a number of extramolecular metals in relatively high concentrations. Of these, at least three—chromium, lead and nickel—are known to be carcinogenic (see Roe & Lancaster, 1964; Furst & Haro, 1969). In one case (Harington, 1965), a single sample of chrysotile was found to contain 5 mg nickel/g fibre (0.5%) and 1 mg chromium/g fibre (0.1%), suggesting that further studies of these metals in this type of asbestos might be profitable. Other metals appearing in relatively high concentration were zirconium, titanium and manganese, in all three types of asbestos studied. Separate experiments have shown that significant amounts of these metals are eluted by serum kept in contact with asbestos for up to two months.

Morgan & Holmes (1970) and Holmes *et al.* (1971), by means of neutron activation analysis, found high levels of chromium, nickel, cobalt and manganese in some samples of asbestos, although it was concluded that the carcinogenicity of different types of fibre might not be related directly to trace metal content *per se* (Morgan & Holmes, 1970).

Apart from being integral or contaminant entities in or on asbestos, carcinogenic metals, for example, nickel, may inadvertently find their way on to fibres during industrial or laboratory milling (Gross *et al.*, 1970).

Morgan & Cralley [1] have summarised the data regarding trace elements associated with asbestos.

Since it was first suggested that trace metals may be implicated in asbestos carcinogenesis, several interesting developments have taken place: (i) Dixon *et al.* (1970) have found that trace metals could inhibit the metabolism of benzo(a)pyrene, increasing the length of time the hydrocarbon might remain in the lung. If this is true, then as Morgan & Cralley point out, the fibre itself would simply be a passive carrier of trace metals; (ii) Cralley (1971) has suggested that a concentration of biologically active cations from fibres could be responsible for carcinogenic action; (iii) Wagner *et al.* (1972[2]) have found high levels of iron, chromium and nickel in samples of Canadian chrysotile, and have concluded that the carcinogenicity of the samples was not related to the content of the three metals mentioned above, nor to

[1] See p. 113 of this publication.

[2] Unpublished data.

cobalt nor scandium. Low carcinogenicity did not seem to be related to low content of trace metals.

(2) *Oils and other organic materials associated with asbestos*

An account of the natural and secondary oils which may be found in asbestos has been given by Harington (1962, 1965). Commins & Gibbs (1969) found that various forms of asbestos, by catalytic action, produce 3,3′,5,5′-tetratertiary butyl diphenoquinone from anti-oxidants present in polythene bags in which asbestos was stored.

Although oils from amosite and crocidolite possess tumour-initiating activity, in comparison with other agents such as 7,12-dimethylbenz(a)anthracene the tumour response seen with the two oils is weak (Roe *et al.*, 1966; Harington *et al.*, 1967). Other findings (Harington, 1965) showed that oil from jute bags (in which asbestos is often transported and stored) is absorbed to an appreciable extent (70–85%) by crocidolite, amosite and chrysotile. Two types of oil used in jute manufacture were found to possess weak carcinogenic activity. On the basis of these results the conclusion was made that the storage of asbestos in jute bags may introduce a significant variable into subsequent determinations of its carcinogenic activity.

Since oil and organic matter are only minor constituents of asbestos, it is difficult to believe that they play a major role in carcinogenesis by asbestos (Roe *et al.*, 1966). Also, although initiating and promoting activities have been satisfactorily demonstrated, this has only been done by using very large doses in relation to the amounts naturally present. The most convincing demonstration that asbestos oils play little or no part in asbestos carcinogenesis is the results of the animal experiments of Wagner & Berry (1969 [1]), and of Wagner *et al.* (1972 [1]). When native (unextracted) UICC asbestos (amosite, anthophyllite, Canadian and Rhodesian chrysotile and crocidolite) and similar samples from which the oils had been removed by repeated extraction with hot benzene were injected intrapleurally into rats, no differences in the numbers of mesotheliomas eventually produced were seen.

At the same time, however, the possibility should be borne in mind that small amounts of carcinogens (for example, hydrocarbons) may be greatly enhanced by the presence of substances of low chemical reactivity such as India ink (Shabad *et al.*, 1964) or iron oxide (Saffiotti *et al.*, 1965). In a different context, Dixon *et al.* (1970) have studied the effects of trace metals on the metabolism of benzo(a)pyrene; and Harington & Smith (1964) have suggested that asbestos in the oil-free state may have either tumour-initiating activity additional to that possessed by the oils, or tumour-promoting activity in its own right. In the latter case, carcinogenicity could possibly arise from asbestos concentrating in the lung on its surface hydrocarbons or other materials which may have entered the lung quite independently of the fibre.

Further valuable data for some type of combined carcinogen-cocarcinogen reaction in asbestos carcinogenesis comes from the work of Miller *et al.* (1965) and has been discussed in detail by Harington *et al.* (1967). Such an action may help to explain why crocidolite generally has a more potent carcinogenic activity than does amosite.

(3) *Asbestos carcinogenesis as an example of tumour induction by chemically inert materials (an "Oppenheimer effect")*

In agreement with Schmähl (1958), it is felt that there is unlikely to be much in common between the mechanism by which asbestos induces cancer and the "Oppenheimer effect" (Harington & Roe, 1965; Roe, 1966). Recent investigations by Stanton [2], however, may lead to a re-evaluation of this matter.

(4) *Contribution of physical attributes*

This aspect has recently been introduced by Timbrell and his colleagues (1971) and may have field applications.

DISCUSSION

The chemical and physical nature of asbestos is complex, so that it would not be surprising if in the induction of cancer by this material several different mechanisms were found to be implicated. The following two possible modes of action are worthy of consideration:

(1) *The asbestos fibre in the pure state* (that is, not contaminated with organic matter or trace metals) may act as the carcinogen, possibly as a macromolecular iron or metal complex, in an analogous

[1] Unpublished data.

[2] See p. 289 of this publication.

way to iron-dextran. Such activity might be investigated by the use of asbestos rendered as pure as possible by solvent extraction; by the removal of trace metals (if this is practical), possibly by the use of synthetic fibre; and by using forms of asbestos with carefully graded contents of iron or other metals.

(2) *Asbestos fibres in the impure (contaminated) state* may act in conjunction: (*a*) with trace metals known to be carcinogenic; (*b*) with oils and other organic matter; (*c*) with both of these; or (*d*) with any radioactivity possessed by the fibres. Carcinogenic trace metals present in asbestos may contribute to the final carcinogenic effect, although it seems unlikely that they could account for the entire effect; this seems to be borne out by the recent studies of Wagner and his colleagues (1972 [1]). Organic matter seems to play a passive or negligible role (Wagner & Berry, 1969 [1]; Wagner *et al.*, 1972 [1]).

The very low values obtained for the radioactive contents of crocidolite, amosite and chrysotile (Harington, 1965) indicate, if anything, that radioactivity plays no more than a very minor role in the induction of cancer by asbestos.

Perhaps as important a consideration as any in work on etiological mechanisms is of the variations in biological activity of the same type of asbestos taken from more than one source. Harington (1972) has recently discussed this matter in detail, pointing out that, in experimental work at least, a standardisation of materials, cells, cell culture techniques and methods of measurement of cytotoxic activity of different forms of asbestos, seems long overdue. The demonstration in haemolysis experiments (Harington *et al.*, 1971b) that heat-labile factors in fresh serum may be required for the activation of biologically weak forms of asbestos, such as crocidolite and amosite, suggests that some factor or factors in the body or around the cell may be required for full expression of activity. The suggested effect of cigarette smoking on asbestos carcinogenesis in man found by Selikoff *et al.* (1973) emphasises the contribution which other extraneous materials may make to the final effect, and such results should not be ignored when *in vitro* experimentation on etiological mechanisms is being planned.

Finally, there remains the obvious yet tantalising possibility that completely new approaches to the mode of action of asbestos as a carcinogen are required, concepts which may be radically different to the limited ones we possess at present. Asbestos has strong adsorptive powers (see, for instance, Macnab & Harington, 1967) which might serve to remove or inactivate cellular constituents from cell surface, cytoplasm or vacuole. Fibres could serve admirably as intracellular micro-ion-exchange columns, or as linear co-polymerising agents, particularly in view of the orderly patterning of cations and anions and the comparative indestructibility of many forms of asbestos.

The elution from asbestos fibres of trace or integral metals, which are known to lie free in the cytoplasm or in phagosomes, might lead to an eventual concentration of such agents in the nucleus, upon its membrane, or on that of other important structures. Such metals might affect DNA or RNA metabolism or alter the properties of membranes by forming ionic bridges; in this respect, important physiological ions such as calcium, potassium and magnesium derived (in excess) from "depot" asbestos in the cytoplasm may have some part to play.

Various types of asbestos possess catalytic properties which affect compounds such as pyridine (Harington, 1965) and, more importantly, physiological compounds like glutathione (Nutt & Harington, 1964); thiol compounds are intimately concerned in cell division (see Harington, 1967b), and their destruction or loss could have an adverse effect on division.

In conclusion, the lack of biochemical studies in which asbestos is used as a carcinogen seems almost complete. Compared to the exhaustive investigations of the carcinogenic polycyclic aromatic hydrocarbons, azo dyes, aflatoxins or alkylating agents, almost nothing has been done with asbestos. Little is known of its effect on enzyme systems or on DNA, RNA or protein synthesis; although it would be surprising if these were not affected, if only because of deletions or changes caused by adsorption or catalysis on or by asbestos. This is borne out by recent studies (Hawtrey & Bester [2]) in which different types of asbestos have been tried as templates for RNA synthesis, with DNA-dependent RNA polymerase added to the system. The radioactive-labelled nucleoside phosphates, adenosine triphosphate, guanidine triphosphate, cytosine triphosphate and uridine triphosphate were adsorbed on to the asbestos samples in varying degrees; in some cases, bind-

[1] Unpublished data.

[2] Unpublished data.

ing was very strong and also time-dependent. The exact effects of these reactions have yet to be evaluated.

Perhaps it is in these comparatively "forgotten areas" of biochemical experimentation that better explanations of the effects of asbestos at the molecular level are to be found.

SUMMARY

Recent studies of lung cancer and mesothelioma have re-opened the subject of the mode of action of asbestos as a carcinogen. In this paper, possible mechanisms of fibrosis and carcinogenesis, and experimental methods of elucidating them, are considered from chemical and biological standpoints. The significance of metal constituents, either as part of the molecular structure of asbestos, or as trace contaminants, and of organic matter present on or in asbestos, is discussed in some detail.

ACKNOWLEDGMENTS

I thank Dr J. C. Wagner, Dr A. Holmes, Dr L. J. Cralley and Prof A. O. Hawtrey for kind assistance, co-operation and for access to unpublished material. Mrs D. Fitzgerald helped considerably with the typing and checking of the manuscript and with the compilation of references.

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Relation of cigarette smoking to risk of death of asbestos-associated disease among insulation workers in the United States[1]

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Data have been reported indicating that cigarette smoking greatly increases the risk of death by lung cancer among asbestos insulation workers (Selikoff *et al.*, 1968). It was calculated that asbestos insulation workers with a history of regular cigarette smoking had eight times the risk of lung cancer deaths compared with cigarette smokers who did not do such work, and approximately ninety times the risk of men who neither worked with asbestos nor smoked cigarettes.

We have obtained further evidence on this matter, bearing on aspects of asbestos-associated disease for which data were previously scant or incomplete.

LUNG CANCER AMONG CIGARETTE-SMOKING ASBESTOS INSULATION WORKERS

Recent experiences have confirmed that lung cancer among insulation workers is largely confined to those men with a history of cigarette smoking. Data are derived from observation of two cohorts of insulation workers. Since they differ in age distribution and work experience, it is advantageous to consider them separately.

First, we have followed a group of 370 insulation workers from 1 January 1963. These were survivors of 632 men who were members of locals of the insulation workers' union in the New York area on 1 January 1943 (Selikoff *et al.*, 1964). Therefore, in 1963 these men were all at least 20 years from onset of employment (indeed, 333 had reached thirty or more years from onset) (Table 1). Two hundred and eighty-three of these men had histories of regular cigarette smoking; by 30 April 1967, 24 had died of lung cancer although, given their smoking habits, only 2.98 such deaths had been expected. No deaths from lung cancer occurred among the 87 men with no history of cigarette smoking (Selikoff *et al.*, 1968).

The cohort has now been traced for an additional 56 months. Table 2 shows findings for the nine-year period from 1 January 1963 to 31 December 1971. Of 283 men who smoked cigarettes regularly, 41 died of lung cancer; while of 87 men who did not smoke cigarettes regularly only 1 died of lung cancer. This man was a cigar smoker. Expected number of deaths shown in Table 2 are based upon United States mortality data for white males, disregarding smoking habits. We are presently unable to calculate smoking-specific expected rates for this group, since death rates related to smoking are not yet available for the period 1967–71[2].

We have obtained data in a second, far larger, study of insulation workers. On 1 January 1967 we registered all members of the insulation workers union in the United States and Canada (including New York–New Jersey locals mentioned above)[3]. There were 17,800 men so enrolled on that day (Table 3). 11,656 completed a questionnaire providing, among other details, information concerning

[1] From the Environmental Cancer Research Project, American Cancer Society and the Mount Sinai School of Medicine of the City University of New York, New York, USA.

[2] We have reported smoking-specific death rates, 1959–65, in a prospective study of 1,000,000 people (Hammond, 1966). This cohort is now being retraced, and rates for 1966–71 will be available.

[3] International Association of Heat and Frost Insulators and Asbestos Workers, AFL-CIO.

Table 1. Members of New York–New Jersey locals of Insulation Workers' Union classified by age as of 1 January 1963, and by years from first occupational exposure to asbestos dust up to 1 January 1963

Age, years	Total no. of members	No. of years since first exposure to asbestos						
		20–24	25–29	30–34	35–39	40–44	45–49	50+
35–39	2	2	—	—	—	—	—	—
40–44	13	12	1	—	—	—	—	—
45–49	32	17	2	13	—	—	—	—
50–54	109	—	1	80	28	—	—	—
55–59	60	—	1	16	34	8	1	—
60–64	42	—	1	3	11	19	8	—
65–69	49	—	—	1	10	18	18	2
70–74	38	—	—	—	3	12	6	17
75–79	21	—	—	—	—	1	5	15
80–84	4	—	—	—	—	1	1	2
Total	370	31	6	113	86	59	39	36

Members classified by age and by smoking habits on or about 1 January 1963

Age, years	Total number	Never smoked regularly	Pipe or cigar only	Ex-cigarette smokers[a]	Current cigarette smokers[a]			
					1–9 per day	10–19 per day	20–39 per day	40+ per day
35–39	2	1	—	1	—	—	—	—
40–44	13	2	—	2	—	—	5	4
45–49	32	2	1	5	—	—	12	12
50–54	109	12	6	26	3	5	33	24
55–59	60	6	5	16	—	3	20	10
60–64	42	7	4	15	1	—	11	4
65–69	49	6	8	17	—	4	9	5
70–74	38	7	7	12	1	4	4	3
75–79	21	3	7	6	—	1	3	1
80–84	4	2	1	1	—	—	—	—
Total	370	48	39	101	5	17	97	63

[a] Includes cigarette smokers who also smoked pipes or cigars.

Table 2. Expected[a] and observed deaths among 370 New York–New Jersey asbestos insulation workers, 1 January 1963–31 December 1971

	Total		No history of cigarette smoking[b]		History of cigarette smoking	
Number of men, 1 January 1963	370		87		283	
Person-years of observation	2520		608		1912	
	Expected deaths	Observed deaths	Expected deaths	Observed deaths	Expected deaths	Observed deaths
Cancer all sites	15.74	94	4.75	15	10.99	79
Lung cancer	4.57	42	1.26	1	3.31	41
Pleural mesothelioma	n.a.[c]	5	n.a.	—	n.a.	5
Peritoneal mesothelioma	n.a.	20	n.a.	7	n.a.	13
Cancer of stomach	0.94	6	0.30	2	0.64	4
Cancer of colon, rectum	2.15	6	0.69	2	1.46	4
Cancer of oesophagus	0.37	—	0.11	—	0.26	—
Asbestosis	n.a.	21	n.a.	5	n.a.	16
All other causes	69.22	53	22.28	15	46.94	38
Total deaths	84.96	168	27.03	35	57.93	133

[a] Expected deaths based upon age-specific US mortality for white males, disregarding smoking habits. Lung cancer estimates based upon US rates for cancer of lung, pleura, bronchus and trachea, categories 162 and 163 of the International Classification of Diseases and Causes of Death, 7th Revision, World Health Organization, Geneva, 1957.

[b] Includes 39 men who smoked pipe or cigars.

[c] United States data not available, but these are rare causes of death in the general population.

Table 3. Membership of Asbestos Insulation Workers' Union[a], 1 January 1967, classified by age and by years from first exposure to asbestos dust

Age, years	Total no. of members	Number of years since first exposure to asbestos								
		0–9	10–14	15–19	20–24	25–29	30–34	35–39	40–49	50+
15–19	244	244								
20–24	1695	1695								
25–29	2412	2066	345	1						
30–34	2762	1065	1356	341						
35–39	2987	313	1140	1342	192					
40–44	2260	79	424	1026	591	139	1			
45–49	1589	49	131	433	442	487	47			
50–54	1297	27	88	214	332	377	182	77		
55–59	983	12	49	129	206	176	146	193	72	
60–64	704	1	21	59	131	126	87	100	179	
65–69	417		6	18	40	57	46	28	200	22
70–74	255			6	14	22	21	16	105	71
75–79	111		1		4	8	4	7	37	50
80–84	52					2	1	2	16	31
85+	32							2	8	22
Total	17,800	5551	3561	3569	1952	1394	535	425	617	196

[a] Membership in the United States and Canada of the International Association of Heat and Frost Insulators and Asbestos Workers, AFL-CIO

Table 4. Smoking habits of 17,800 Asbestos Insulation Workers in the United States and Canada, on 1 January 1967

Age	Total	No history of cigarette smoking[a]	History of cigarette smoking	Smoking history not known
<25	1939	281	782	876
25–29	2412	285	1182	945
30–34	2762	314	1435	1013
35–39	2987	309	1640	1038
40–44	2260	223	1395	642
45–49	1589	172	964	453
50–54	1297	134	821	342
55–64	1687	201	965	521
65–74	672	122	314	236
75+	195	25	92	78
Total	17,800	2066	9590	6144

[a] Included 609 men who smoked pipes or cigars.

their smoking habits (Table 4). We have followed this cohort through 31 December 1971 (Selikoff *et al.*[1]). Although the total group differed from the cohort described above in being, on the average, significantly younger and with shorter duration of exposure, its lung cancer experience has been very much the same.

1,092 deaths occurred during the period 1 January 1967 to 31 December 1971 (see Table 5). Of these, 213 were due to lung cancer; whereas only 44.4 were expected had the experience of these men been the same as that of other US white males of the same age distribution. Among the 9590 men with a history of regular cigarette smoking, there were 596 deaths, 134 of which were due to lung cancer. Again, we are at this time unable to calculate smoking-specific expected and observed rates because, as noted, death rates related to smoking habits of individuals are unavailable for this period of years.

LUNG CANCER DEATHS AMONG INSULATION WORKERS WHO DO NOT SMOKE CIGARETTES

At the time of our initial report, we had had limited opportunity for studying the incidence of lung cancer among insulation workers with no history of cigarette smoking. There were 87 such men

[1] See p. 209 of this publication.

Table 5. Expected[a] and observed deaths among 17,800 US and Canada Asbestos Insulation Workers, 1 January 1967–31 December 1971

	Total		No history of cigarette smoking[b]		History of cigarette smoking		Smoking habits not known	
Number of men, 1 January 1967	17,800		2,066		9,590		6,144	
Person-years of observation	86,300		10,163		46,615		29,522	
	Expected deaths	Observed deaths	Expected deaths	Observed deaths	Expected deaths	Observed deaths	Expected deaths	Observed deaths
Cancer all sites	144.09	469	19.92	33	79.58	265	44.59	161
Lung cancer	44.42	213	5.98	2	25.09	134	13.35	77
Pleural mesothelioma	n.a.[c]	26	n.a.	2	n.a.	17	n.a.	7
Peritoneal mesothelioma	n.a.	51	n.a.	9	n.a.	29	n.a.	13
Cancer of stomach	6.62	16	0.95	1	3.60	8	2.07	7
Cancer of colon, rectum	17.51	26	2.52	4	9.53	14	5.46	8
Cancer of œsophagus	3.21	13	0.44	0	1.80	7	0.97	6
Asbestosis	n.a.	78	n.a.	4	n.a.	45	n.a.	29
All other causes	661.54	555	92.67	36	356.67	286	212.20	233
Total deaths	805.63	1092	112.59	73	436.25	596	256.79	423

[a] Expected deaths based upon age-specific US mortality rates for white males, disregarding smoking. Lung cancer estimates based upon US rates for cancer of lung, pleura, bronchus and trachea, categories 162 and 163 of the International Classification of Diseases and Causes of Death, 7th Revision, World Health Organization, Geneva, 1957.
[b] Included 609 men who smoked pipes or cigars.
[c] United States data not available, but these are rare causes of death in the general population.

in our 1963 New York–New Jersey group, and by 1967, only 16 deaths had occurred, none of lung cancer. Only 0.18 lung cancer deaths were expected, however, and with such scant experience we concluded that our information "... does not prove that exposure to asbestos dust has no influence on the risk of lung cancer among non-smokers. However, it does suggest that exposure to asbestos dust does not lead to an extremely high risk of lung cancer among non-smokers." (Selikoff *et al.*, 1968.) Obviously, it was important to obtain further information on the lung cancer risk among non-smoking insulation workers. This is now available, from experience of the cohort described above.

Among the 2066 non-cigarette smokers in the nation-wide study, 73 deaths occurred between 1 January 1967 and 31 December 1971. Two were due to lung cancer. One of these two men was a cigar and pipe smoker, and the other never smoked regularly (Table 5).

It seems clear, then, that lung cancer is uncommon among asbestos insulation workers who have no history of cigarette smoking, and that if the risk is increased such an increase is not great.

PLEURAL MESOTHELIOMA

In our previous report, we were unable to suggest whether or not pleural mesothelioma was related to cigarette smoking. Only three deaths from this disease occurred in our New York–New Jersey group between 1963 and April 1967. While all three of these men were cigarette smokers, the number was too small for reliable evaluation. Since then two more deaths from pleural mesothelioma have occurred, again among cigarette smokers (Table 2).

In the larger cohort (see Table 5) there were 1092 deaths, of which 26 were due to pleural mesothelioma. Of these 26 men, 17 had a history of regular cigarette smoking, 1 was a pipe smoker, 1 never smoked regularly and 7 were unknown as to smoking habits. We still refrain from drawing definite conclusions because of the small numbers involved.

PERITONEAL MESOTHELIOMA

As with pleural disease, no definitive statement could be made in 1968 concerning the relation of peritoneal mesothelioma to cigarette smoking. Of seven deaths due to peritoneal mesothelioma, two occurred among men with no history of cigarette smoking.

In the large cohort (see Table 5) there were 51 deaths from peritoneal mesothelioma, 9 among the 2066 who did not smoke cigarettes regularly, and 29 among the 9590 cigarette smokers. Thirteen

deaths occurred among 6144 insulation workers for whom smoking histories were not available (Table 5).

These experiences suggest that cigarette smoking does not increase the already high risk of peritoneal mesothelioma among asbestos insulation workers.

ASBESTOSIS

Studies indicate that radiologically-evident pulmonary fibrosis is augmented in asbestos workers by cigarette smoking (Weiss, 1971).

Data now at hand suggest that the risk of death from asbestosis (respiratory insufficiency and cor pulmonale) may be increased by cigarette smoking. These data are reported with the recognition that there must be a mixture of effects of cigarette smoking in such cases, including increased asbestotic fibrosis, and the emphysema, bronchitis and smoking-associated fibrosis related to cigarette smoking in general (Auerbach *et al.*, 1963). These effects could be additive to different degrees, or multiplicative, in specific cases; complex histological and physiological variations are possible.

In the nation-wide study, of the 73 deaths among the 2066 non-smokers, 4 were due to asbestosis, as were 45 of the 596 deaths among the 9590 smokers (Table 5). We computed expected numbers of asbestosis deaths from age-specific death rates for the total study population, disregarding smoking habits.

The ratio of observed to expected asbestosis deaths was almost three times as high for men with a history of cigarette smoking as for men without a history of cigarette smoking. This was of borderline statistical significance.

GASTRO-INTESTINAL CANCER

There seems to be a definite, albeit limited, association between employment in asbestos insulation work and increased risk of death from cancer of the stomach, colon-rectum and oesophagus. Data in this regard were first reported in 1963 (Selikoff *et al.* 1964).

Experiences since 1963 continue to indicate the same conclusion, with increased death rates of approximately the same magnitude. In the large cohort (see Table 5) there were 16 observed *versus* 6.62 expected deaths from cancer of the stomach, 26 observed *versus* 17.51 expected deaths from cancer of the colon-rectum, and 13 observed *versus* 3.21 expected deaths from cancer of the oesophagus. Because of the small numbers of expected and observed deaths from cancer of these sites among the 2066 men with no history of cigarette smoking, we will draw no conclusion concerning the possible interaction of cigarette smoking and asbestos exposure. However, these data are consistent with findings in other studies of a high degree of association between smoking and the occurrence of cancer of the oesophagus.

SUMMARY

We conclude that employment in asbestos insulation work greatly increases the lung cancer risk of cigarette smokers. It is uncertain whether such employment increases the risk of lung cancer among non-smokers. Cigarette smoking may also increase the risk of death from asbestosis, although to a much lesser extent. It is of interest that the risk of death among non-smoking asbestos insulation workers is greater for asbestosis than for lung cancer. This indicates that even if asbestos workers were to stop cigarette smoking, it would still be necessary to reduce dust exposure below those concentrations associated with the occurrence of asbestosis.

ACKNOWLEDGMENT

This research was supported in part by US Public Health Service grant OH00320 to the Mount Sinai School of Medicine.

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Discussion summary

A. MORGAN [1]

The Chairman opened the discussion by relating the results of epidemiological and experimental investigations presented at this meeting. He pointed out that the evidence suggests that both asbestosis and bronchial carcinoma appear to be unrelated to fibre type and that there is a dose-response relationship, with the most heavily exposed groups most at risk. In contrast, the production of mesothelial tumours in man seems to be closely linked with exposure to crocidolite and may be a chance event in relation to the amount of fibre in the lung. Animal experiments, in which various types of asbestos were administered by intrapleural inoculation, showed that in these circumstances chrysotile was as effective as crocidolite in producing mesothelial tumours. This apparent anomaly between the experimental and epidemiological findings can be explained by assuming that small crocidolite fibres, when inhaled, penetrate more readily to the pleural cavity. Consideration of the dimensions of the crocidolite fibre indicate that it is probably the most respirable type of asbestos, and its chemical stability ensures its persistence in lung tissue. It was pointed out that nothing is known regarding the initiation of mesothelial tumours. The properties of the mesothelial cell were compared with those of the macrophage, and some indications were given as to why it may be more susceptible to carcinogenic change.

The first two papers described the results of animal experiments in which asbestos and glass fibres, aluminium oxide whiskers and non-fibrous materials were administered to experimental animals by a number of routes. Some questions were asked about the preparation of these materials. It was suggested that the reduction of fibres by ball milling could produce lattice distortion, and that the surface energy of materials prepared in this way may be quite different from that of the original. A brief account of the technique used in preparing the samples for Dr Stanton's experiments indicated that every precaution had been taken to avoid such effects. No measurements of trace elements in these materials had been made, however. Dr Wagner agreed that the brucite used in his experiments may have contained intergrowths of chrysotile fibrils. Additional experiments were described by Dr Davis in which glass fibre and crocidolite had been injected intraperitoneally into rats and mice. Tumours were produced by both these materials, the first indication being the formation of tiny nodules on the surface of the peritoneum, some of which are attached to the wall of the body cavity. Initially, each nodule is covered by an intact layer of mesothelial cells, but eventually this layer may be disrupted. Further work is planned to elucidate the significance of these findings.

In the third paper, Dr Timbrell described how a study of apparent paradoxes had led to the discovery of factors of major importance in the etiology of asbestos diseases. He suggested that a similar systematic approach might assist in identifying the most probable of the proposed mechanisms of asbestos carcinogenesis. Because of the enormous numbers of fibres in the lungs of occupationally exposed groups, the initiation of tumour production could be an improbable event by normal standards. He stressed the importance of developing techniques for the accurate assessment of fibre in lung tissue.

The importance of chemical factors in the etiology of asbestos carcinogenesis was considered by Dr Harington in the fourth paper. He discussed the significance of trace metal and organic contaminants of asbestos and suggested that it may be worth considering a "micro-Oppenheimer" effect. In subse-

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quent discussion, modification of the carcinogenicity of Ni_3S_2, administered to rats by intramuscular injection, was described by Dr Cralley. The simultaneous injection of equimolecular amounts of elemental chromium and manganese appeared to reduce the carcinogenicity of this material. It was suggested that this phenomenon could be accounted for on the basis of electromotive interactions of these metals.

After the final paper, there was a contribution from Dr Vosamäe, who described experiments in which chrysotile asbestos and 3,4-benzpyrene were administered to rats by intratracheal injection, both separately and in conjunction. The results showed that chrysotile enhanced the carcinogenicity of the polycyclic aromatic hydrocarbon.

Unfortunately, time did not permit detailed discussion of the paper by Drs Hammond and Selikoff. After the session, however, there was some comment on the data given in Table 5 of this paper for the expected death rates due to lung cancer. As the authors state in the text, values for the smoking specific death rates for individuals in the general population were not available for the period under review at the time of writing. As the expected death rates in Tables 2 and 5 disregard smoking habits the data in these tables should be interpreted with caution.

ASBESTOS AND THE COMMUNITY

Chairman — J. C. Gilson

Rapporteur — G. Berry

Industrial uses of asbestos

K. V. LINDELL [1]

Much has been published in the medical journals concerning the geology, occurrence, types, mining, milling and manufacture of asbestos, thus these aspects will not be discussed here. Some mention has also been made of the industrial uses of asbestos, but the great number of these uses precludes any complete report on this subject in a paper which must be limited in length. However, there are some points made in previous presentations which require elaboration, and the "why" of the need of asbestos fibre in the world we live in should be presented. A brief explanation of the grading of asbestos fibre is given, and a listing of the characteristics of the principal fibres used, together with some important comparisons.

CLASSIFICATION OF ASBESTOS FIBRE

Asbestos fibre is often described as being graded according to length. Actually, no grade of asbestos has any one specific length, but is classified according to those fibres remaining upon a set of screens after a standard period of agitation. The Quebec Standard Testing machine has four boxes, with screen openings of 0.500 in., 0.187 in., 0.053 in., the fourth being a receptacle for the fines which fall through the screens. A one-pound sample of asbestos is agitated for 600 revolutions at 328 rpm, and the resulting fractions are weighed. The fibre is sold on a guaranteed minimum test of the various fractions.

To further illustrate the length variation, Tables 1, 2, and 3 show the general characteristics of the principal grades of amosite, crocidilite, and chrysotile, with their main uses. Most fibres contain fines which will pass through a 200 mesh screen, although

Table 1. Amosite grades

General classification	Grade			McNett test		Surface area cm^2/g	Main use
	Penge mine	Welte-vreden mine	Kromel-lenboog mine	+14 M %	−100 M %		
Extra long	D.11			70	20	3000	Moulded insulation
Long	M	W3	K3	64	26	3500	Moulded insulation, general
	S11			62	26	3500	insulation and board products, asbestos cement pressure pipes
Medium	S22	SW	SK	59	26	4000	Insulating and fire resisting
	S22/65			40	35	8500	boards, refractory tiles
Short	S33	GW	GK	46	35	5500	Insulating and fire resisting
	S33/65			33	40	8500	boards, refractory tiles and
	S44			18	50	8500	asbestos cement
			RK	21	47	6000	Asbestos cement sheets and
			6805	10	55	7000	moulded products, plastic reinforcement

The values shown in the above table are typical test results and are not to be used as specifications.

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Table 2. Crocidolite grades

General classification	Grade	McNett test		Surface area cm^2/g	Main use
		+14 M%	−100 M%		
Long	C	94	4	1,000	Asbestos textiles
	A	90	7	2,000	Felts for reinforced plastics
Medium	S	67	26	6,500	
	S80	62	30	10,000	Asbestos cement pressure pipes
	2S	58	30	7,000	
	H	50	33	8,000	
	H80	36	43	10,000	Asbestos cement sheets
Short	C10	30	49	14,000	Asbestos paper
	WDS	33	43	9,000	Asbestos cement sheets

The values shown in the above table are typical test results and are not to be used as specifications.

Table 3. Chrysotile[a]

General classification	Grade	McNett test		Surface area cm^2/g	Main use
		+14 M%	−200 M%		
Long	3R–3K	76	18	10,600	Asbestos textiles
Medium	4T–4K	43	31	7,700	Asbestos cement, pipes and sheets
Medium-short	5R–6D	20	44	8,900	Asbestos cement sheets, paper, friction materials
Short	7D–7R	4	69	11,800	Tile, friction materials, caulking, putties

[a] Typical Canadian chrysotile.

there are some fibres produced today that have negligible fines. These fines usually contain both fibrous and non-fibrous material; the ratio of fibrous to non-fibrous fines varies from one asbestos type to another. It would be useful to determine the proportions of respirable fibres in the various grades.

TEXTILES

Perhaps one of the oldest uses of asbestos fibre is in the manufacture of textiles. The flexibility and strength (Table 4) of asbestos, and above all its resistance to fire (Table 5) provide the world with a most unusual and useful product. From the legendary story of Charlemagne's "magic table-cloth" to its multitude of uses in our modern economy, asbestos has led the way in protecting man under the most hazardous conditions. Similarly, because of its resistance to physical, electrical and heat effects, the textile applications of asbestos range from wire and cable insulation to uses in motors and transformers. Since asbestos has exceptionally good resistance to nuclear radiation, to fungus, and to length reduction upon processing, it is superior in textile uses to almost all of the other mineral fibres.

Table 4. Comparison of tensile strengths of various materials

Type of material	Tensile strength (lbs/in^2)
Ingot iron	45,000
Wrought iron	48,000
Carbon steel	155,000
Ni-Cr steel	243,000
Piano steel wire	300,000
Cotton fibre	73,000 to 89,000
Rock wool	60,000
Glass fibre	100,000 to 200,000
Chrysotile asbestos	450,000
Crocidolite asbestos	500,000
Amosite asbestos	350,000
Tremolite asbestos	<75,000
Anthophyllite asbestos	240,000

Table 5. Effect of heat on tensile strength of Canadian chrysotile (crude)

	Tensile strength (lbs/in^2)	Per cent of original tensile strength
Original crude—no heat	400,000	—
Heated 3 min at		
600°F	120,000	91.6
800°F	96,000	73.3
1000°F	78,000	59.5
1200°F	42,000	32.0

Asbestos yarns braided into various configurations have been used highly successfully as packing materials in pumps, pistons and so on; such braided asbestos products treated with a variety of saturants are extremely durable under rugged conditions of use. In such applications the resilient, soft, non-abrasive characteristics of asbestos are put to good use in keeping essential machinery operating efficiently and at low cost.

For the conditions prevailing in an internal combustion engine, no other fibre is as effective as is chrysotile asbestos in the mixtures used; it resists the attack of hot gases, hot oils, steam, hot water and anti-freeze mixes; and it is more resilient than other fibres—efficient jointing-sealing materials must be resilient. There is no conceivable health risk in the use of asbestos-based gasket materials.

The total amount of asbestos fibre used in the textile industry is relatively small compared to the total tonnage consumed. It is, however, an important use, since it also provides protective clothing and insulation covering for conditions where satisfactory substitutes have not yet been found. Also available today is a dust-suppressed cloth, made of yarn produced by a wet dispersion process.

FRICTION MATERIALS

Man's need for transportation is as old as civilisation itself; but, as his need for rapid transportation has increased, so have the perils he has brought upon himself. Next to fire and war, stopping motion represents perhaps mankind's greatest self-inflicted danger.

The ideal brake-lining has not yet been compounded. We know that it must have a high coefficient of friction for braking but that it must not score the brake-drum. It must be able to withstand the high temperatures which occur when the high energy of motion is transformed into heat; and it must not pass this heat inward or it would destroy itself too quickly. It must be ready to be used again, on short notice; all this without noise or other pollution. It has been shown that asbestos does provide good braking, without scoring the brake-drum. It disintegrates (only on the surface) to a powder, so that a new surface is presented immediately. It is ductile and soft enough to do all this without noise and with only a minute loss of material (material which has been transformed to an amorphous substance and is no longer a fibre). It does not permit the heat to be transferred inward to destroy other materials which may have been used as binders for the compound. It has proved strong enough to resist all the handling involved in its manufacture up until the stage at which it becomes a part of the finished brake-lining. It wears slowly; and above all permits brake-linings to be made economically and reproducibly time and again to demanding specifications, in order to ensure the utmost in safety in cars and in other moving vehicles in use today.

It is not surprising, then, that asbestos is a vital part of the composition of friction material. Its high strength provides a toughness necessary for suitable and dependable service, without any apparent risk to the general public.

Studies by the United States Public Health Service show that only a tiny fraction of the asbestos in brake-linings is released to the air as airborne particles in their original form as the brakes wear. Alternative reinforcements, such as glass fibre, have lower strength, low melting-points and cause damage to the brake-drum; steel wool has low availability, low strength, and causes damage to the drum; mineral wool has low strength and is brittle to the extent of limiting mixing processes. Other alternative materials, such as sintered metals and ceramics, have inconsistent behaviour at various temperatures; heat transference creates a risk of the brake fluid boiling. Asbestos fibre is the only reinforcing fibre which imparts consistent performance to friction linings and which provides frictional stability and fade resistance.

PAPER PRODUCTS

Asbestos paper products, ranging in thickness from a few millimetres to approximately $\frac{1}{4}$-inch, serve to protect man and his property in many ways. Because of their electrical resistance such products are used as insulation in transformers, electrical motors and as wire or cable wrappings. Their resistance to heat allows them to be used as protective layers in high-temperature devices such as furnaces, heaters, etc. When saturated with asphalt, asbestos papers provide a protective roofing which does not rot and which is extremely weather resistant. Used as a roofing underlay, asbestos felt provides protection against disastrous fires such as those which swept the West Coast of the United States a few

years ago. Their weather- and rot-resistance and their compatibility with asphalt allow asbestos felts to be used as protective wraps on metallic pipe-lines, which are otherwise subject to corrosive attack when buried underground.

While all of the man-made mineral fibres possess the non-burning characteristic of asbestos, none other of them possesses the ability to attract and retain binders and saturants as does chrysotile asbestos. The unique positive charge of chrysotile attracts and retains the negatively charged binder particles to the felted product. According to Woolery (1966), this characteristic of chrysotile asbestos also enhances the retention by cellulose papers of filler and pigment, thereby effecting a material cost reduction in one of the world's largest and most important industries.

ASBESTOS CEMENT PRODUCTS

Regardless of his location on this earth, man's need and search for adequate shelter and respite from the elements is rivalled only by his need and search for food and water. Asbestos cement products provide the world's population with safe, long-lasting, weather-resistant homes and places of work; their resistance to fire, weather, rot, rust or oxidation and destruction by rodents and insects is unparalleled among building materials. Asbestos has a great contribution to make to housing in developing countries, since the cement industry, which relies largely on indigenous materials, is always one of the first to get underway in a developing country. The introduction of an asbestos cement plant can, therefore, with the least disruption to the economy, produce large quantities of sheet for the roofs and walls of buildings. In developed countries, the use of such sheets remains an economic means of covering factories and buildings; and since there is no health risk involved in the handling of this type of material for this purpose it should continue to fulfil an important role in construction.

Simularly, asbestos cement pipes help to meet man's demand and need for water by providing strong, non-corroding, economical conduit systems; their smooth bore is maintained since they do not become encrusted. Asbestos is used in such products for several reasons among which are its high strength, its compatibility with cement systems, its ability to form felts or webs from aqueous slurries, and its resistance to cement alkalis and to physical attrition under manufacturing conditions.

Since the uses of asbestos cement are so widespread, it is not surprising that considerable effort has been made to find substitute materials which might be cheaper, or which could be found indigenously in the countries of manufacture. To date, such programmes have not been productive, or, at best, only partially successful. It is important therefore to assess those properties of asbestos which are responsible for its unparalleled performance in the construction industry.

Asbestos fibres have extremely high tensile strengths, which in most cases far exceed those of any other fibrous material. Zukowski & Gaze (1959) have shown that the varieties of asbestos used in asbestos cement products have average tensile strengths ranging from 440,000–600,000 lbs/in^2; they indicate in fact that under the most favourable circumstances, and with very short fibres, tensile strengths of nearly 900,000 lbs/in^2 may be obtained.

In the manufacture of asbestos cement products, cement is retained by asbestos fibres by both physical entrapping and chemical absorption. According to Biwas (1962) and Chatterji & Dhangal (1961), chrysotile fibre retains a greater amount of cement by chemical absorption than do the amphiboles; however, this property of chrysotile asbestos should be considered only a contributing attribute rather than the prime factor in the resulting high mechanical strengths of asbestos cement products. For example, tremolites provide only about 50 per cent of the transverse strength in asbestos cement sheets, probably due to the much lower tensile strength of this variety of asbestos and its lower absorption of cement. On the other hand, the particular structures of amosite and crocidolite cause these fibres to provide more bulk volume in both the dry state and in aqueous suspension than does chrysotile; and their surface properties have a vital effect in any manufacturing process employing a wet system. In asbestos cement manufacture, amphiboles complement chrysotile by improving the drainage rate and by dispersing the chrysotile fibres more effectively.

Most asbestos cement products are formed from wet slurry systems; because of their thin diameters and their flexibility asbestos fibres in an aqueous slurry felt together easily to form a web, which in turn entraps and retains the cement particles. Fibres such as mineral wool, basalt wool and glass wool are spicular in shape, have lower surface areas

Table 6. Solubility of asbestos

Per cent loss in weight after re-fluxing for two hours in 25% acid or caustic

	HCl	CH_3COOH	H_3PO_4	H_2SO_4	NaOH
Actinolite	20.31	12.28	20.19	20.38	9.25
Amosite	12.84	2.63	11.67	11.35	6.97
Anthophyllite	2.66	0.60	3.16	2.73	1.22
Chrysotile	55.69	23.42	55.18	55.75	0.99
Crocidolite	4.38	0.91	4.37	3.69	1.35
Tremolite	4.77	1.99	4.99	4.58	1.80

Per cent loss in weight at room temperature (26°C) for 528 hours in 25% acid or caustic

	HCl	CH_3COOH	H_3PO_4	H_2SO_4	NaOH
Actinolite	22.55	12.14	20.10	20.60	9.43
Amosite	12.00	3.08	11.83	11.71	6.82
Anthophyllite	2.13	1.04	3.29	2.90	1.77
Chrysotile	56.00	24.02	56.45	56.00	1.03
Crocidolite	3.14	1.02	3.91	3.48	1.20
Tremolite	4.22	1.41	4.89	4.74	1.65

and do not have the felting and cement retention characteristics so well demonstrated by asbestos. For these reasons, presently known manufacturing processes for cement products can utilise only limited amounts of these artificial fibres in place of asbestos without seriously and dangerously impairing the strength and quality of the product.

Alkaline conditions evolving in cement systems do not attack asbestos fibres (Table 6); this is not true of many of the synthetic or artificial mineral fibres. Slag wool fibres have a poor resistance to alkali, as do glass fibres of normal composition; and as a result asbestos cement products made with these types of materials deteriorate in strength with time. Recent publications discuss the potential use of special alkali-resistant glasses in cement products; however, the present cost of such fibrous materials far exceeds the performance:cost ratio of asbestos in the largest portion of the asbestos cement product line. The alkali-resistant glasses have not yet been tested for a period comparable to the normal life of an asbestos cement sheet; and it may well be that glass fibre suffers degradation several years earlier than does the sheet, an occurrence which could have dangerous consequences.

Finally some mention must be made of the resistance of asbestos to particle size reduction under all conditions of processing. Asbestos fibres are extremely resistant to length attrition, this being particularly true of the chrysotile variety. The brittle character of artificial fibres such as mineral wool, glass and others is well known; these materials cannot withstand the mixing, fiberising, pulping and slurry transport processes which are common to most industries. In this respect asbestos has few, if any, rivals in the field of reinforcing fibres (Table 7).

Table 7. Comparison of approximate fibre diameters

Type of fibre	Fibre diameter in micrometres	No. of fibrils in one linear inch
Human hair	40	630
Ramie	25	1015
Wool	20–28	900 to 1250
Cotton	10	2500
Rayon	7.5	3300
Nylon	7.5	3300
Glass	6.5	3840
Rock wool	3.5–7.0	3250 to 7040
Asbestos (chrysotile)	0.018–0.030	850,000 to 1,400,000

OTHER EQUALLY IMPORTANT USES

A complete story of the "why" of asbestos would need to include its indispensability in asbestos-reinforced thermosetting plastics, and similar uses, all for the same reasons.

As a further comment on substitutes, it should be pointed out that since we cannot be sure that the dust from some of the materials now being advocated as substitutes for asbestos materials is not the cause of latent disease, there is a strong argument for the use of materials for which, on the basis of a half a century's experience, a standard has now been established which enables us to prescribe the conditions under which they can be used with safety.

CONCLUSION

The industrial use of asbestos has unfortunately in a number of cases been accompanied by a record of industrial disease. Even though in many countries the development of dust control systems has considerably reduced this hazard, it is only in comparatively recent years that it has been appreciated that the handling of some of these materials may be hazardous. There is not time or space in this paper to discuss this aspect; however, it should be recognised that much progress has been made in developing methods of control which make it possible to continue to enjoy the benefits of this unique material without undue risk to the health of those who work with it.

SUMMARY

The paper attempts to present the "why" of the need for using asbestos. The classification of asbestos fibres and their general characteristics are discussed. Tables are included to demonstrate these characteristics.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the assistance of the following in the preparation of this paper and in supplying the up-to-date information for the charts: W. C. Streib, Johns-Manville Corporation; Dr Richard Gaze and A. A Cross, Cape Asbestos Company Limited; W. Howard, Asbestos Information Council. The final editing was done by John Gossip of the Quebec Asbestos Minining Association.

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The 1969 (UK) Asbestos Regulations—their economic appraisal

R. L. AKEHURST [1]

As the British Occupational Hygiene Society sub-committee on asbestos (BOHS, 1968) pointed out, "Knowledge of the relationships between airborne dust exposure and the risk of asbestosis is not in itself sufficient to establish a hygiene standard. Another important problem, and one which is very difficult to resolve, is that of balancing the risks to health against the consequences of demanding excessive dust reduction." The problem that committee faced was the same as would be faced by a group trying to frame hygiene standards for any substance—to decide, given the best information available on the technical relationships involved, how much regulation was required. This is exactly the kind of problem of choice with which economics is concerned. An investigation of the effects of the 1969 Asbestos Regulations will indicate the consequences of setting the Regulations at their existing level and shed light on the nature of the problem to be faced when other substances have to be controlled.

Accordingly, this paper describes research, at present being undertaken at the University of York, into the impact of the 1969 Asbestos Regulations on the UK. It sets out: (1) the aims of the research and the conceptual difficulties involved in carrying it out; (2) the practical difficulties that arise in moving to actual valuation, and the approaches used to deal with them; and (3) the progress made to date and the outstanding problems.

THE AIMS OF THE RESEARCH

The research has the following aims: first, to assess the total costs and benefits accruing to the inhabitants of the UK as a consequence of the introduction of the 1969 Regulations; second, to estimate the distribution of those costs and benefits, i.e., to see which groups in the economy gain and which lose and by how much; and third, to examine how these costs and benefits might vary if any change in the Regulations were made.

If the results are to be useful, values for costs and benefits must be directly comparable, that is they have to be in the same units. Clearly, most costs are in money [2]; but benefits can be in the form of money, disability averted, and lives saved. The problem that has to be faced is how much money is equivalent to x disabled men or y dead men. The problem is particularly acute when disability is dealt with, because degree of disability varies. A number of approaches to valuation of death and disability have been suggested in the economic journals, none of which is entirely satisfactory. However, it should be noted that some values must be placed, either implicitly or explicitly, and that despite the difficulties involved consistency is more likely to be achieved if the valuations are explicit.

ESTIMATION OF BENEFITS AND COSTS

Identification and evaluation of benefits

The benefits which arise from the introduction of the 1969 Regulations all come from one common root—the reduction in the number of people who will contract asbestos-related diseases. Estimates are therefore required of the number of people who would have been expected to contract asbestosis or

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[2] Although there are circumstances in which disability and death could appear on this side of the balance.

an asbestos-related cancer if the 1969 Asbestos Regulations had not been introduced, and of the equivalent number now that they have been introduced. The expected benefits of the Regulations stem from the difference between these two estimates.

The assumption is made that dust levels which existed before the introduction of the Regulations would have continued unchanged but for that introduction. The data necessary to calculate the expected incidence of asbestos-related disease are therefore: (i) *the population at risk*; (ii) *their expected cumulative exposure in the absence and presence of the Regulations*; and (iii) *the relationship between cumulative exposure to asbestos dust and incidence of disease.*

(i) *The population at risk* is very poorly identified because the use of asbestos is so widespread. The best population estimate available was published for the year 1963 in the Department of Employment and Productivity (1968) Memorandum, although the Department itself admits that it could be a serious underestimate, since persons who might be exposed by reason of working near where asbestos is manipulated are excluded. The asbestos manufacturing firms have agreed to supply information on the rates of growth, turnover and re-entry of workers in the industry, and, using this information together with the Department's figure, a projection for the exposed population will be obtained.

(ii) *The expected cumulative exposure* to asbestos dust before the present regulations were introduced is very difficult to estimate. The main difficulty stems from the wide variation in exposure levels. There are some data available which relate to, probably, the extreme cases. These are dust counts for the naval dockyards at Devonport (Harries, 1971), and counts for the Turner Brothers factory at Rochdale published by the BOHS (1968). Estimates for pre-Regulations dust counts can only be made on the basis of this information, and with the advice of experts in the asbestos industry. An attempt was made to obtain information on dust counts from HM Factory Inspectorate, but without success. Estimation of post-regulation dust levels provides no difficulty as these are set by the Regulations, and it is assumed they are complied with.

The second element in cumulative exposure is the length of time of exposure. The asbestos manufacturing firms have agreed to provide information on the average length of stay of workers in the industry. This information, together with that on the rates of turnover and re-entry, enables estimates to be made of the length of exposure of the workers and thus of their cumulative exposures.

(iii) Establishing *a relationship between cumulative exposure and incidence of disease* is not simple, because the relationships between exposure and incidence, especially for the cancers, are not well known. Two published works will be used for this study. The first was published by the BOHS (1968) and gives the relationship between cumulative exposure to chrysotile asbestos and the incidence of asbestosis, as indicated by the presence of basal rales. This enables an estimate to be made of the expected future incidence of asbestosis. The second work was published by Newhouse (1969) and is not strictly comparable with that by the BOHS, since the latter considered men who had only been exposed to chrysotile, while the former also included men exposed to crocidolite and amosite. From Newhouse's work, standardised mortality ratios (SMR's) will be obtained for cancers and respiratory infection for workers who have been exposed to certain (roughly) known levels of mixed asbestos fibre. From the tables of Case *et al.* (1968a, b), the number of people expected to die in a sample of the general population of the same size as the exposed population will be calculated. Any excess of deaths that would be expected as a result of asbestos exposure will be calculated by applying the SMR's to this figure.

Once the numbers of people who would be expected to contract an asbestos-related disease in the presence or absence of Regulations have been calculated, the value of any reduction attributed to the Regulations has to be estimated. Benefits will be of three types: (i) *The removal of the cost of treating people* who would have been expected to contract asbestosis or cancer; (ii) *the reduction of working-time lost* through asbestos-related disease; and (iii) *The saving of life and the avoidance of disability* per se.

(i) *Costs of treatment.* Cancers and asbestosis present different problems. No useful figures on the cost of identifying and treating cases of cancer (particularly lung cancer) are available, but the problem is made more simple because the prognosis for the asbestosis-related cancers is so poor. Very few sufferers recover, and often the time from diagnosis to death is very short. Most of the treatment cost involved is therefore incurred in terminal care. Estimates of the average time from diagnosis to death and time in hospital will be obtained from the medical profession. The cost per week of treating an in-

patient can be obtained from the Hospital Costing Returns of the Department of Health and Social Security (1971); and these two pieces of information will be used to calculate the cost of treating a cancer sufferer.

The problem is more complex with asbestosis, because, typically, the sufferer lives on for many years after diagnosis with the disease gradually becoming more disabling. No information is available on the pattern of use of health service facilities by asbestotics in comparison to their healthy colleagues, and in order to obtain this information (together with other data necessary for this study) a survey was initiated in Devonport (Harries, 1971). It will provide the physical pattern of use of health service facilities by asbestotics and this will be costed by a variety of data, the most important of which will be the Hospital Costing Returns, 1971.

(ii) *Work loss.* Work losses by cancer sufferers are relatively small. Workers who die from asbestos-related cancers are often retired; and if they are not, they leave work when their illness becomes too severe for them to carry on working, and never return. The period of work loss for those who are not retired will be treated as coincident with terminal care.

By contrast the asbestotic often stays on at work for many years, perhaps taking increasingly more sick-leave, until he is forced to take a lighter job, part-time work or to retire because of illness or age. Data are being collected in the Devonport survey on work loss and this will be costed on the assumption that the cost of time lost, in all but long-term disability cases, is the product of the wage rate and the number of hours of work lost.

(iii) *Valuing death and disability* per se. As explained earlier, some value has to be placed on death and disability. The figures to be used in this study have been carefully derived, but the method used for the derivation can be explained satisfactorily only at some length. A paper outlining the method, as yet unpublished, will be available shortly (Akehurst & Culyer, 1972 [1]).

Identification and evaluation of costs

The costs of introducing the 1969 Regulations may appear in three forms: (i) Those costs arising because firms which manufacture or use asbestos products take *physical precautions* to diminish the health hazard. An example is the installation of new exhaust equipment. (ii) Those costs arising because erstwhile consumers of asbestos goods switch to *substitute goods.* These costs may arise either because more money has to be spent on the substitute to achieve a given technical performance, or because the standard of performance is allowed to drop. (iii) Those costs which result from an asbestos product being completely withdrawn from production with no substitute being available. So far, no evidence of costs of this third type arising has been found, and this category is not, therefore, discussed further. Costs of this type might, however, become important if the Regulations were made more stringent.

(i) *Costs of physical precautions.* In order to estimate the extent of these costs the major manufacturers of asbestos products have been asked how much they spent on changes made in response to the regulations. Specifically, they were asked the capital cost of installing any new equipment, rooms, laundries, etc., made necessary, and the running costs of these new additions. Further, they were asked to estimate what the cost would be of complying with even more stringent regulations. This information the manufacturers have agreed to supply. However, to obtain a full picture of the costs in this category it is necessary to find out what steps the consumers of asbestos goods have taken. A difficulty here lies in identifying consumers—many asbestos products are sold to wholesalers who then sell to the actual consumers. Also, the manufacturers, for commercial reasons, are not willing to disclose who their customers are. Two large consumers, the Central Electricity Generating Board and the Admiralty, have been approached for assistance, and it is to be hoped that their co-operation will be forthcoming.

(ii) *Substitution costs.* The only way in which these costs can be estimated is by directly approaching the consumers of asbestos goods. Information was requested from the manufacturing firms on sales to major customers in recent years in order to identify which firms had changed their purchases. It was intended that the manufacturing firms would then provide an introduction to certain of their customers and indicate what proportion of the total relevant market they took. The manufacturers will not, however, release sales data nor provide introductions, and so far no figures have been obtained in this cost category.

[1] Unpublished data.

PROGRESS TO DATE AND PROBLEMS OUTSTANDING

At the time of writing sufficient information has been promised or is being collected to enable the benefits expected from the 1969 Regulations to be estimated. Also, this same data will make possible an estimate of health benefits (or costs) of any further change in the level of dust control. There is, of course, a large measure of uncertainty involved in the estimate and it is proposed to calculate a probability distribution instead of a single figure for benefits. However, this will take time and was not ready by October 1972.

Information has also been promised on the cost to the manufacturers of asbestos products of the introduction of the Regulations and any change in their stringency. This is useful and interesting information, but it is certainly not sufficient to provide a cost figure comparable to the benefit figure. To obtain even a roughly comparable figure, time, and the co-operation of some of the major consuming firms, will be necessary.

No mention has been made of the second aim of the research—an estimation of the distribution of the costs and benefits of the Regulations. In part, the problems here are considered in the discussion of the total impact, but not entirely. For example, work loss is considered in the estimation of the total effect, but compensation is not. The reason is that work loss involves *loss* of output, whereas compensation involves only the *transfer* of money from a body to an individual. However, both are important to the worker as an individual, and therefore both have to be considered when we turn to the distributional impact of the Regulations. Among the data, not already mentioned, necessary for assessing distributional impact are: disaggregated profit figures; the pricing policies of manufacturing firms; compensation paid to workers; and the effect on the work-force of changes in sales. The manufacturing firms have declined to release any of this information, so prospects for progress in this area are not good. Nevertheless, some of the data could be obtained from other sources.

In the immediate future, however, work will be directed to completing the estimate of the overall impact of the Regulations.

SUMMARY

The paper outlines the data that must be collected and the problems that are involved if the effects of the 1969 Asbestos Regulations on the UK are to be evaluated. Costs and benefits arising from the introduction of the Regulations are identified, and the means by which they can be evaluated are indicated.

ACKNOWLEDGEMENTS

Acknowledgement is made to the Medical Research Council and the Asbestosis Research Council for grants to the Institute of Social and Economic Research in the University of York for research into this topic.

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Discussion summary

G. BERRY [1]

In presenting his paper, Mr Akehurst was able to give some information recently obtained from the survey in Devonport. Comparing workers with asbestosis and controls, those with asbestosis lost on average £150 per year due to having to transfer to a less arduous job, £180 per year due to having to take more time off work due to illness and £600 per year due to premature retirement. The extra cost of using the health service was small compared with the above. Thus asbestosis costs about £900 per year per case and this does not include any estimate of the cost of disability *per se*.

The discussion which followed the two papers in this session concentrated on two points: first, to what extent industry could modify its processes to use the less dangerous types or components of asbestos, and secondly, whether the difficulties of placing values on health and life meant that the economic approach was inevitably doomed to failure.

On the first point, it was reported that the substitution of chrysotile for crocidolite in the manufacture of asbestos cement pipes had led to problems initially but that these had now been overcome, so that crocidolite was no longer essential for this purpose. However, the use of chrysotile leads to the production of micro-fibres, and it is not known whether it will be possible to prevent this. Some industrial processes give products in which the fibre is "locked in" and therefore no longer a danger. On the other hand, production of finer chrysotiles (grades 6 and 7) has increased in recent years.

In the discussion on health economics, it was observed that nothing was indispensable any more than anything was wholly safe. Allowance might be necessary for lives lost through not using asbestos, if increased costs led to a reduction of its use. But, if an equally effective substitute were available, it would not be necessary to make any such allowance, although the extra cost of using the substitute would have to be taken into account.

There are dangers in restricting an economic appraisal to a single country. For example, strict regulations could lead to an "export" of the more dangerous industrial processes, so that the health hazard was not eliminated but simply transferred to another group of workers. The present economic analysis did not take account of such possibilities, but did take account of the distributional effects among different groups of individuals within the United Kingdom.

The difficulty of placing a value on health and life was fully recognised but this did not mean that the question could be ignored. The application of health economics had not introduced the problem but rather pointed out that it existed. The introduction of policies or regulations which results in an increase in the costs of providing necessary products to the community in return for a reduction in the hazard to health of those occupationally exposed to asbestos, implicitly at least, places an economic value on health and life. By the application of economic methodology, the valuation would become explicit, enabling the consequences of any decisions to be discussed realistically by those involved.

It was emphasised that the economic approach was only being suggested as a means of evaluating the effects of policies which were applied to groups of people, and was certainly not intended to be applied by the clinician who was treating individuals who had developed disease.

As Mr Akehurst's study was the first attempt to apply economic principles to the regulation of the use of a material, there were inevitably great difficulties, but the methodology developed could be expected to be applicable in other fields.

[1] MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, UK.

ASPECTS REQUIRING FURTHER STUDY

Chairman — P. Bogovski

Rapporteurs — J. C. Wagner, V. Timbrell

Report on an informal session

J. C. WAGNER [1] & V. TIMBRELL [1]

This Session was devoted to aspects which participants considered had not been covered in the agenda.

STUDY OF IMMUNOLOGICAL ASPECTS OF MESOTHELIOMAS

It was considered that an immunological approach would be valuable in the understanding of the development of diffuse mesotheliomas in asbestos-exposed populations. If it could be shown that the tumours produced specific antigens, these might be used for the surveillance of populations at high risk. Eventually, this might lead to immuno-therapeutic methods. There was a great need to develop a method of treatment in cases of mesotheliomas, as none of the present forms of therapy influence the prognosis. The lapse between exposure to asbestos dust and the development of these tumours was on average 40 years. This indicated that even if all significant exposure to asbestos dust was immediately eliminated, asbestos-related mesotheliomas will continue to the end of this century.

Various methods of immunological study were suggested. The only results available, of a preliminary nature, were presented by Dr J. S. P. Jones. He reported that Professor R. W. Baldwin's team in Nottingham, UK had produced some evidence for a cell-mediated immunity in mesotheliomas. They obtained the mesothelioma cells from pleural effusions, lymphocytes from cases with mesotheliomas and samples from control subjects. It was urged that further investigations of this type should be undertaken on serum and cells from workers at high risk; these should be compared with matched controls and patients with definite tumours.

[1] MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, UK.

There was a need to obtain living cells from mesothelial tumours. The ethics of asking surgeons to excise large biopsy specimens from patients were discussed. This was thought justifiable in certain selected cases, where full preparations had been made for the culture, preservation and use of the tissue. In this peculiar tumour it was of paramount importance to ensure that the cells growing in culture are actually derived from mesothelial cells; this required electron-microscopic examination of the tissue. It was recommended that serum and lymphocytes be obtained from populations at high risk. These could be preserved for later study; the serum should be frozen and the lymphocytes specially preserved in liquid nitrogen.

EDUCATION OF THOSE EXPOSED TO ASBESTOS

Although it was agreed that the education of workers on the possible risks associated with exposure to asbestos was not within the terms of reference of the Working Group, considerable concern on this aspect was felt by many of the participants. Prevention was the best means of control. Information was available to workers in the primary industry, but in some countries the majority of subsidiary users were thought to be quite unaware of the possible hazards. It was recommended that the views of the Working Group should be brought to the attention of those concerned with implementing all aspects of prevention. The extra hazard of cigarette smoking to those exposed to asbestos dust should be widely recognised.

TUMOURS OF THE GASTRO-INTESTINAL TRACT

The presence of an increased incidence of cancers of the gastro-intestinal tract in asbestos workers had

been shown in several studies. The excess risk was less than for lung cancer, but more investigations were needed to discover if the organs contained asbestos. The studies starting in Sweden could provide further evidence on this as the cancer registry was excellent, and a preliminary investigation had shown that half the members of the insulation and construction unions were non-smokers.

MATERIALS

It was agreed that the UICC reference samples had been of great use. Although such samples could not represent the changes which were continually occurring in the fibres produced by different areas, they did provide a valuable basis for relating biological effects to physical and chemical properties. Further samples would be useful for determining the range of variation known to exist in the characteristics of the same type of fibre from different sources.

In view of new observations, that other fibres as fine as asbestos could also produce mesotheliomas in experimental animals, glass and other fibrous materials should be studied in detail. Interpretation of results would be simplified if fibres were available in narrow ranges of diameter and length. Methods for preparing such graded materials should be developed and checked for their effects on the physical properties of the materials.

MANGANESE

There was some discussion on the possible importance of manganese in relation to the mesotheliomas observed in the crocidolite mining area of the north-western Cape Province of South Africa. It was pointed out that as a result of changing the beaters in the mills, the manganese content of the fibre had been substantially reduced. The change had been made subsequent to the preparation of the UICC crocidolite sample, which could not therefore be considered representative of present production. Doubt was expressed about the relevance of manganese. There were considerable variations in the manganese content of the strata of individual mines and in fibre from different sources. Some coals contained up to 50,000 ppm of manganese but no increased incidence of tumours had been reported. There was at present no direct epidemiological or experimental evidence strongly pointing to its importance.

STANDARDISATION OF ASSESSMENT METHODS

Many discussants stressed the need for standardisation of methods used to assess asbestos fibres in air samples and in tissue. There was strong support for the suggestion that exchange of samples be arranged. Use of this approach had effected substantial improvement in coal mine studies. It was suggested that the IARC might establish a Panel to study such problems.

REPORT OF THE ADVISORY COMMITTEE ON ASBESTOS CANCERS TO THE DIRECTOR OF THE INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

Report of the Advisory Committee on Asbestos Cancers to the Director of the International Agency for Research on Cancer

The meeting was held at the International Agency for Research on Cancer, Lyon, France on 5 and 6 October 1972. The Committee consisted of three Panels—Epidemiology, Pathology, and Physics and Chemistry.

Epidemiology Panel

Dr M. Becklake; Dr H. Bohlig; Dr N. Day; Prof P. C. Elmes; Dr J. C. Gilson (Chairman); Dr J. Lepoutre; Prof J. C. McDonald; Mr C. E. Rossiter; Dr H. Sakabe; Dr I. J. Selikoff*; Dr G. K. Sluis-Cremer; Dr W. Smither; Dr G. Wright.

Pathology Panel

Dr A. C. Allison; Mr G. Berry; Dr P. Bogovski; Dr M. Kannerstein; Prof W. T. E. McCaughey; Prof D. Magner; Prof H. Otto; Dr H. T. Planteydt; Dr M. Stanton; Dr J. C. Wagner (Chairman); Prof S. Watanabe; Prof I. Webster.

Physics and Chemistry Panel

Dr L. Le Bouffant; Mr G. W. Gibbs; Dr S. Holmes; Mr A. Morgan; Dr F. D. Pooley; Dr S. Speil; Dr V. Timbrell; Dr R. du Toit; Mr W. H. Walton (Chairman).

The Panels met in separate sessions and at the final session (Chairman, Dr J. C. Gilson) prepared this report to the Director of the International Agency for Research on Cancer.

TERMS OF REFERENCE

1. The Committee was to report on the present evidence relating exposure to asbestos dust to cancers, especially that obtained since the meeting of the UICC Working Group on Asbestos Cancers in 1964.

2. The Committee was to make recommendations for further research and indicate priorities for work of immediate and long-term value.

CO-ORDINATION OF INTERNATIONAL CO-OPERATION

Following the meeting of the UICC Working Group on Asbestos and Cancers in 1964, a Sub-Committee of the UICC Commission on Geographical Pathology and Environmental Carcinogens (Chairman, Dr J. Higginson) was formed to co-ordinate work required to achieve the recommendations.

In April 1970 agreements between the UICC and the IARC led to the winding-up of the UICC Sub-Committee and to the IARC taking on responsibility for the Sub-Committee's work and extending it by supporting certain projects on asbestos cancers in several countries. The Agency has done this as part of their wider programme of investigating environmental carcinogens. Common memberships between the UICC Sub-Committee and the Committee advising the IARC ensured continuity of policy.

In October 1972 the IARC held an international conference with 137 participants from 20 countries to review all the evidence relating asbestos with cancers. Subsequently the Advisory Committee prepared its report. This is divided into two sections. First, a general review in the form of answers to a number of important general questions about the relation of asbestos to cancers of different sites and, secondly, recommendations for further research.

* Dr Selikoff did not attend and later dissociated himself from the Report of the Advisory Committee.

GENERAL REVIEW

1. Are all major commercial types of asbestos able to cause lung carcinoma?

Yes. Since 1964 the evidence of a causal relationship has been increased by epidemiological studies showing exposure-response relations for the incidence of lung carcinomas. The production of lung carcinomas in certain animals by all types of asbestos supports this conclusion. The epidemiological evidence in man, however, shows that there are clear differences in risk with type of fibre and nature of exposure.

2. Is there evidence of an increased risk of lung carcinoma at low levels of exposure to asbestos, such as have been encountered by the general population in urban areas?

The evidence of an exposure-response relationship based in part on past dust measurements and in part on the type of job within the industry suggests that an excess lung carcinoma risk is not detectable when the occupational exposure has been low. These low occupational exposures have almost certainly been much greater than that to the public from general air pollution.

3. Since 1964 has the evidence relating past exposure to asbestos and mesotheliomas changed?

The evidence has been greatly strengthened by further prospective and retrospective mortality studies in many countries of populations exposed to asbestos. There is evidence that all commercial types of asbestos except anthophyllite may be responsible. Evidence for an important difference in risk in different occupations and with the type of asbestos has increased. The risk is greatest with crocidolite, less with amosite and apparently less with chrysotile. With amosite and chrysotile there appears to be a higher risk in manufacturing than in mining and milling. There is also evidence from population studies that a proportion of cases of mesothelioma have no known association with exposure to asbestos.

4. Is there evidence of an increased risk of mesothelial cancers at low levels of exposure to asbestos, such as have been encountered by the general population in urban areas?

There is evidence of an association of mesothelial tumours with air pollution in the neighbourhood of crocidolite mines and of factories using mixtures of asbestos fibre types. The evidence relates to conditions many years ago. There is evidence of no excess risk of mesotheliomas from asbestos air pollution which has existed in the neighbourhood of chrysotile and amosite mines. There are reported differences on incidence of mesothelioma between urban and rural areas, the causes of which have not been established. There is no evidence of a risk to the general public at present.

5. Since 1964, has the evidence changed on the importance of other factors, such as cigarette smoking, waxes, oils and trace elements, as contributory factors to the cancer risks?

The evidence has accumulated indicating:

(1) Cigarette smoking is an important factor enhancing the lung carcinoma risk in asbestos-exposed workers, in both men and women. Asbestos workers have especially strong grounds for giving up smoking to protect their health. No association has been demonstrated between cigarette smoking and mesotheliomas.

(2) Animal experiments designed thus far to test the importance of waxes and oils as contributory factors in the production of mesothelioma have shown these contaminants are unlikely to be relevant.

(3) From animal experiments there are no good clues suggesting that trace elements are likely to be a major factor in the production of asbestos cancers.

6. What other types of cancer are related to exposure to asbestos?

Prospective surveys of occupational groups exposed to asbestos have in general shown a small excess risk of some other types of cancers (in addition to bronchial and mesothelial), especially those of the gastro-intestinal tract. The excess of these tumours is relatively small compared with that for bronchial cancer. Evidence for an association with ovarian tumours has not been supported by the first large mortality survey of women previously exposed to asbestos.

7. Is there evidence of an increased risk of cancer resulting from asbestos fibres present in water, beverages, food or in the fluids used for the administration of drugs?

Such evidence as there is does not indicate any risk.

8. Is there evidence of a risk of lung fibrosis from low levels of exposure to asbestos such as have been encountered by the general population in urban areas?

There is at present no evidence of lung damage by

asbestos to the general public. The amount of asbestos in the lungs of members of the general public is very small compared to those occupationally exposed. It is greatest where asbestos is mined or worked, and lowest in rural areas.

9. Has the relationship between asbestos exposure and the development of pleural plaques been established?

Pleural plaques have been associated with past exposure to all commercial types of asbestos. But additional factors, other than asbestos itself, are involved. The plaques may remain fibrous or become calcified. Not all pleural plaques are associated with asbestos.

RECOMMENDATIONS FOR FURTHER RESEARCH

Projects which the Panels rated high in priority are marked *; those which will require close co-operation between the Panels are marked †.

EPIDEMIOLOGY

The Panel agreed that asbestos-related cancers occur in several sites in the body. The incidence of the different cancers varies with a number of definable factors and for other reasons, such as competing causes of death. Epidemiological studies will usually provide information on more than one type of cancer. Research directed at only a single type may, on occasions, be useful but in general the inevitable uncertainties, in some cases in the differential diagnosis of, for example, peripheral lung carcinomas and pleural mesotheliomas, and between peritoneal mesotheliomas and other intra-abdominal cancers, will require that more than one type is studied at the same time.

The Panel recognised that some of the epidemiological projects could only be pursued if there was close co-operation between epidemiologists, pathologists, physicists and chemists, and others, because their success will depend upon the development of improved techniques, some of which are referred to in the recommendations of the other two Panels.

PROJECTS

(1) Further development of objective methods for early detection and surveillance of effects caused by asbestos. Topics for particular study include:

(*a*) Immunological techniques for screening for fibrosis and neoplasia.
(*b*) Functional tests of changes in the peripheral airways.
(*c*) Detection of pleural thickening.
(*d*) Assessment of the specificity of small irregular opacities in the chest radiograph as defined in the ILO-U/C Classification, 1971.
(*e*) Tests of the usefulness of different techniques of chest radiography, including the use of 100 mm films.
(*f*) Development of statistical procedures for analysis and presentation of serial observations.

† (2) Evaluation of the usefulness of early detection in the prevention of progressive fibrosis and asbestos cancers, also in the identification of hazardous conditions. Routine health surveillance of industrial populations should be designed to assist epidemiological studies and should include measurement and recording of environmental dust levels. Surveillance of new entrants could be particularly valuable. Arrangements should be made to register workers, so that their morbidity and mortality experience can be studied even after cessation of exposure to asbestos.

* (3) Assessment of excess cancer risks following exposure to only one type of fibre.

(*a*) *Chrysotile:* The much higher cancer risk reported for chrysotile textile workers compared with mine and mill workers requires explanation. How much is explicable by differences in size of airborne fibres and past dustiness? There is need to make more use of past dust records for relating to indices of disease.
(*b*) *Amosite:* The excess lung carcinoma and mesothelioma risk is apparently much greater in the manufacturing and application sections of the industry than in the mining and milling of this type of fibre. What are the important factors in this reported difference?
(*c*) *Crocidolite:* Further studies are required in occupational groups exposed only to crocidolite or amosite or chrysotile in the manufacturing and application parts of the industry to establish more clearly differences in risks due to different fibres.

* † (4) Studies of the amount and type of asbestos in the lungs of cases of mesotheliomas (if possible by cell type) in (*a*) national survey of mesotheliomas,

(*b*) representative samples of cases arising in groups with a definable past exposure.

* (5) Studies of secular changes in incidence of pleural and peritoneal mesotheliomas nationally and internationally.

* (6) Epidemiological studies to investigate the association between past exposure to asbestos and cancer of sites other than lung, pleura and peritoneum.

(7) Studies of secular trends in the asbestos content of the lungs in the general population.

* † (8) Studies to relate amount and type of asbestos in the lung and estimates of past dust exposure and interval since last exposure.

(9) Experimental and epidemiological studies to investigate possible differences of effect of continuous low and intermittent high exposure to asbestos.

(10) Opportunities afforded by intercurrent deaths should be used to interrelate radiographic appearances, lung pathology, respiratory function, dust content and type in asbestos workers. Standardised techniques and classification recommended by the Panels should be used.

(11) Investigate the prognostic significance and etiological factors in the development of calcified and uncalcified pleural plaques in different environments.

(12) Investigate talc-exposed groups in mining and manufacturing to establish any differences in morbidity or mortality which might be related to the amount and shape of the fine respirable particles.

(13) Development of cost/benefit analyses to study the health, safety, social and economic interrelations of the use of asbestos.

PATHOLOGY AND EXPERIMENTAL PATHOLOGY

The Panel reviewed the progress made on the 1964 UICC recommendations. It was agreed that considerable progress had been made on the majority of the recommendations. Some require further study, or modification of previous methods of investigation; these are included in the list of recommendations that follows. The recommendations are divided into three categories, morbid anatomy and histology, clinical research and experimental studies.

PROJECTS

Morbid Anatomy and Histology

1. Asbestosis

* (1) Further consideration should be given to methods for determining the amounts, types and structural features of asbestos in tissue. A Sub-Committee should be established with members of the Physics and Chemistry Panel, and others, to accelerate work on this problem.

(2) The methods for assessing the severity of asbestosis [1] should be tested for consistency by different observers.

2. *Carcinoma*

* † (1) An investigation of whether reduction of asbestos exposure to levels below those producing asbestosis also abolishes excess risk of carcinoma was considered important.

(2) A comparison of lung carcinomas in persons occupationally exposed to asbestos and those not so exposed, including both cigarette smokers and non-smokers, in respect of sites of origin and cytology of tumours and presence or absence of asbestosis, would be of value.

3. *Mesotheliomas*

(1) The International Panel of Pathologists [2] and National Panels established following the 1964 meeting [3] have served a useful purpose. It is recommended that Panels be established in other countries and membership of the International Panel be extended. The main purpose of these Panels is to ensure uniformity of diagnostic criteria and recording of histological types of diffuse mesotheliomas. Collaborative study of histology slides in National Panels is recommended. The diagnosis of mesothelioma can be made by exfoliative cytology of the pleural fluid; if the cytological diagnosis is made by a competent cytologist, biopsy may be unnecessary.

(2) To improve consistency of diagnosis there is an urgent need for a Comprehensive Atlas on meso-

[1] See p. 54 of this publication.

[2] The International Panel consists of: Dr M. Kannerstein (USA); Prof D. Magner (Canada); Dr L. Meurman (Finland); Prof W. T. E. McCaughey (Eire); Prof H. Otto (FRG); Dr H. T. Planteydt (Netherlands); Dr E. Roitzsch (GDR); Prof L. Santi (Italy); Prof I. Webster (South Africa); and Dr J. C. Wagner (UK) as Secretary.

[3] Great Britain, South Africa, United States, Canada, The Netherlands.

theliomas, or alternatively, for inclusion of an enlarged section on mesotheliomas in the new edition of the WHO Monograph on Tumours of the Lung. Criteria for diagnosis by exfoliative cytology and a description of the fine structure of mesotheliomas should be included.

Clinical Research

* † (1) Monitoring by immunological methods of populations exposed to asbestos should be investigated to ascertain whether it is possible to recognise those who are developing, or will develop, tumours.

(2) The use of chromatographic methods for the study of mucopolysaccharides and other tumour-associated substances in pleural fluids should be explored. Sensitive methods might be developed and applied to identify secretory products of mesotheliomas in blood and urine.

Further Experimental Studies

* (1) Information is required about the role of fine particles, especially influence of fibre size, in the induction of tumours. These studies should be extended to include fibres other than asbestos. A Sub-Committee should be established to review the need for, and arrange the distribution of, standard samples of asbestos and other fibres in addition to the UICC reference samples.

† (2) The fate of inhaled particles of various sizes, shapes and chemical compositions should be studied to determine more precisely the quantities and sites of initial deposition, change within the body and later retention. The feasibility of increasing fibre elimination by various methods should be explored. Studies should be made of means of reducing the fibrogenicity and carcinogenicity of fibres already retained in the lungs.

(3) The use of cell and organ culture, including mesothelial tissue from man and other species, should be further investigated with a view to developing methods of screening dusts for fibrogenic and carcinogenic properties.

(4) Further studies should be carried out to determine the nature of the combined effect on tumour induction when animals are exposed to asbestos dust and cigarette smoke, metals or other chemical carcinogens, including those which act systemically such as nitrosamines.

(5) Inhalation experiments should be extended to test various types of fibre; of special interest are forms of chrysotile and crocidolite including the finer grade materials.

(6) It was felt that studies of the pathological effects of asbestos on species other than rodents would be of value.

(7) The effect of long-term ingestion of fibres of various sizes, shapes and chemical compositions should be studied.

(8) The effects of fibres and associated metals on the metabolism of target organs should be investigated.

PHYSICS AND CHEMISTRY

The Panel reviewed the progress made on the 1964 UICC Recommendations. The proposals for the preparation and characterisation of the UICC reference samples of asbestos had been satisfactorily implemented, and the Panel recommended that a list of references to papers featuring the samples should be distributed to investigators in this field. Considerable progress had been made on methods of identifying the type of fibre in tissues, but a quantitative method when several types of fibres were present had yet to be developed.

The Panel discussed the further contribution that physical and chemical studies can make to research on the biological effects of asbestos and other fibrous materials. Of especial interest are the effects of fibre size and shape on the retention of material in the lungs, the site of deposition, the migration of fibres within the body and their carcinogenic or other biological activity. The following recommendations were made:

PROJECTS

* † 1. *Materials for experimental work*

(*a*) Supplies of asbestos from relevant sources should be obtained where there is evidence of variation in geological form, trace element content or significant biological findings.

(*b*) Small samples of various fibrous materials should be prepared for studies on the influence of fibre size and shape on carcinogenicity. For this purpose the samples should be milled to different degrees of fineness.

(*c*) For investigations on the influence of particle shape and size on the inhalation and subsequent fate

of asbestos fibres, a chrysotile and an amphibole of fibre length greater than the UICC samples should be prepared.

* † 2. *Methods*

(*a*) There is an urgent need for the quantitative assessment, size analysis and characterisation of particles and fibres in the lungs and other organs. Details of available methods should be circulated, international comparisons undertaken, methods standardised and new techniques developed.

* † (*b*) No methods are at present available for the preparation of fibres in narrow ranges of diameter and length in sufficient quantities for inoculation experiments. Techniques for these purposes are urgently required, especially in view of the advantages such graded samples could provide for investigating the influence of these physical factors on the carcinogenicity of fibres of different materials.

(*c*) Since the degree of dispersion of fibres (especially chrysotile) used in inoculation studies may have a marked influence on their carcinogenicity, methods are required for quantifying dispersion.

(*d*) Inhalation studies require precise control of the characteristics of the dust clouds. Improved methods of dispensing fibrous dusts in such investigations need to be developed.

(*e*) Methods are available for collecting the important size fractions of dust clouds in inhalation studies when the particles are of compact shape. Similar methods must be developed for fibrous particles.

(*f*) The present membrane filter methods of measuring the levels of airborne asbestos dust require standardisation. This should be done by inter-laboratory trials on a continuing basis. Particle counting by electron microscopy should also be developed. Gravimetric assessment methods and the automation of particle counting should be explored.

† 3. *Inhalation studies*

Considerable information is now available on the deposition, retention and migration of particles of compact shape. Recently developed methods, especially radioactive tracer techniques, should be used to obtain similar knowledge for fibrous particles. This information is needed to identify the biologically important size fraction and to help interpretation of epidemiological and pathological studies.

4. *Occupational and environmental studies*

The use of both fibre counts and gravimetric methods for assessing asbestos dust concentrations should be encouraged. Data collected over an extended period will be particularly valuable in identifying the parameters of the dust which can be correlated with epidemiological evidence on the health hazard.

5. *Physics and Chemistry Panel*

* It is recommended that an International Panel be established to assist in implementing these recommendations. The Panel would periodically review requirements for materials for experimental work; provide guidance on physical and chemical problems; and arrange national and international standardisation trials.