THE RETENTION AND CLEARANCE OF INHALED GLASS FIBRE AND DIFFERENT VARIETIES OF ASBESTOS BY THE LUNG

Roderick Edward Geoffrey Rendall

A dissertation submitted to the Faculty of Medicine, University of the Witwatersrand in fulfilment of the requirements for the degree of Master of Science.

JOHANNESBURG 1988

ABSTRACT

The inhalation of fibrous dusts draws many millions of fibres into the lung where they may cause damage and disease. Some fibres enter the lung more easily than others and some are more readily removed than others. In this study the lungs of baboons which had been exposed to different types of fibre were examined and the build up of fibres in the lung after different periods of exposure plotted. Similarly the clearance of fibres from the lung after exposure was determined.

Significant differences between glass fibres and asbestos fibres were found. The clearance of the fibres was expressed in terms of a 'half life'. The half lives varied from 6 months for glass fibre to 50 months for crocidolite asbestos. This is a possible factor in the development of lung disease following extended exposure to fibrous dust.

DECLARATION

I declare that this dissertation is my own unaided work. It is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

R.E.G. RENDALL

23rd day of MARCH 1988.

ACKNOWLEDGEMENTS

I am pleased to acknowledge the assistance and encouragement I have received from the following people while preparing this dissertation.

Firstly I thank Prof. J.C.A. Davies, Director of the National Centre for Occupational Health, where the work was done, for constant genial encouragement.

Next Mrs. M. Colly who is in charge of the animal house, who cared for the animals while they were being exposed to dust and during their survival period after exposure and finally, when they died, removing their lungs. This was the base material for the study.

Then members of my own department especially F.S.J. Coetzee, who is in charge of the dust exposu e facility who supervised the dust exposures and maintained meticulous records of the dust concentrations and G.C.H. van Sittert who carried out the electron microscopy.

A special acknowledgement is due to Enoch Mogomotsi who carried out the bulk of the physical work in cutting up the samples of lung tissue, digesting them and counting the fibres extracted under the microscope.

Lastly my thanks go to Dr. R.S.J. du Toit who estimated the dust exposures of the miners which were used in the miners' lung clearance graph.

Finally I thank Drs. Malcolm Steinberg and Peter Barron for fruitful discussions and advice on the Statistics chapter and for use of the NANOSTAT Statistical package on the computer.

Other people who assisted significantly with the production of the work were A. McInnes of the Photography Department and Mrs. V. Poplak and her staff of the Library.

One last word of appreciation goes to Mrs. Lorraine Kotzé who typed and retyped the various drafts which the work went through before completion.

R.E.G. Rendall

SUMMARY

This work was carried out to investigate the relative rates of clearance of different types of inorganic fibre from the lungs after exposure to high but known doses of these fibres. The material used was lung tissue from baboons which had been exposed to fibres in the dust inhalation facility of the National Centre for Occupational Health in the course of other investigations. The work is covered in 12 Chapters and is best summarized by considering each of these chapters in turn.

The first chapter, Chapter 1 touches briefly on the importance of the work and, by taking a specific example, highlights some of the deficiencies in much of the work which has been done in the field. This is more fully covered in Chapter 2 which is a review of published work.

The materials used are described in Chapter 3, firstly according to their mineralogical or chemical composition and then according to their comparative physical characteristics i.e. the length, diameter, and size distribution of their component fibres.

In Chapter 4 the actual conditions under which the dust inhalation took place are described especially the dust concentrations of the different fibres used and how they were controlled. This Chapter also includes the inhalation and survival histories of the 56 baboons involved in the study. It was not possible to work on tissue from all these baboons, as some were diseased at death and others are still alive.

Chapter 5 describes the instruments used for measuring dust concentrations, their purposes, principles and limitations.

In Chapter 6 the method used to extract the fibres from the lungs is described. This is an elaborate and lengthy procedure as precautions have to be taken to ensure that the fine fibres in the tissue were not destroyed or broken. In either of these events the counts obtained would not be a true reflection of the number of fibres in the tissue.

Chapters 7 and 8 are concerned with the light microscopy and electron microscopy of the airborne and extracted fibres. Electron microscopy is necessary because the diameter of many of the fibres is equal to or less than 0,3µm the limit of resolution of the light microscope. The factors which determine the limit of resolution of light and electron microscopes are briefly discussed.

It was inevitable that a scientific computer would have to be used to sort and present the considerable amount of data generated in this investigation. The system used, and the software generated, is described in Chapter 9. The importance of being able to programme the computer to do exactly what is required rather than to rely on a commercially available programme is stressed.

In Chapter 10, the most important chapter, the results of 4 investigations are presented graphically. It is from the graphs that the final results of the work were obtained. These show significant differences in the rates of clearance of the different types of fibre. Crocidolite asbestos is shown to have the slowest rate of clearance with

a half life of 50 months, next is amosite with a half life of 15 months and glass fibre, 6 months. It was not possible to determine a half life for chrysotile because of the high mortality among the animals during the exposure period. These half lives are in the same order as the fineness of the fibres and their generally accepted toxicity.

Chapter 11 deals with the statistics of the dust exposures and the clearance patterns observed. It is concerned mainly with the confidence limits which should be applied to the various means quoted and the significance of the trends. In the final chapter, Chapter 12 the limitations of the study are discussed together with the more interesting aspects of the findings.

A list of references is attached which indicates the wide range of interest in the subject. Finally examples of the forms used to collate the data, computer programs and comparative data from human material are included in a series of appendices.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

NOTE : FIGURE NUMBERS ARE THE SAME AS THE TEXT PARAGRAPH NUMBERS TO WHICH THEY REFER

LIST OF FIGURES

NOTE : FIGURE NUMBERS ARE THE SAME AS THE TEXT PARAGRAPH NUMBERS TO WHICH THEY REFER

CHAPTER 1

INTRODUCTION

This study is based on the lungs of a large number of baboons which had been exposed to different types of fibrous dusts in the dust inhalation facility at the National Centre for Occupational Health (NCOH). The period of exposure of each baboon was known as was the mean dust concentration to which it had been exposed. Furthermore its survival after removal from the exposure chamber until its death could be established. The inhalation and retention in the lung of inorganic fibres such as asbestos or glass fibre is a prime cause of industrial lung disease. The most serious of these is a rare form of cancer of the pleura, known as mesothelioma. Since this was first clearly associated with the inhalation of asbestos fibres by Wagner in 1963[1] the subject of fibre retention and clearance has received much attention. The relationship between fibre inhalation and the development of disease is a complex one involving the length and diameter of the fibres[2] and their time of residence in the lung. Of these factors the most potent appears to be the period of residence $(t)^3$ in the lung whose effect may be proportional to t^3 or t^4. As the period of residence is related to the rate of clearance of dust from the lung the latter may be an important factor affecting the potential hazard of such inhalation.

In this study the main theme has been the mathematical relationship between the number of fibres found in the lung after different periods of survival. Little reference has been made to the degree of disease

induced by the dust or other causes. Previous work at the centre has shown that all baboons which have been exposed to asbestos for more than two years have a marked degree of asbestosis while in the case of those exposed to similar concentrations of glass fibre the pathology in the lung is minimal or very slight.[4] Mesotheliomas began to appear in some baboons exposed to crocidolite or amosite fibres 4 or 5 years after removal from the exposure chambers but this reaction was considered to be outside the scope of this study as it is not related to the clearance of fibres.

Clearly two routes of investigation are possible. The first is to examine the lungs of deceased miners and others who have worked with asbestos during their life and the second is by animal experimentation. Both methods have their advantages and disadvantages. The human lungs are the chief focus of interest but it is difficult to estimate the dose of asbestos or other fibre which a man has received during his life. With animal experiments it is possible to measure the dose inhaled fairly accurately but since the animal lung does not have exactly the same structure as the human lung it may not react in the same way. To a certain extent this latter disadvantage may be overcome by using non-human primates whose lungs are more similar to human lungs than are those of the smaller experimental animals. This is what was done in this study.

Many studies involving other animals have been reported and these are discussed in Chapter II but though they have been most elegantly designed and meticulously executed they do not really relate to the conditions under which humans inhale fibres in real life. A typical

protocol involves a short sharp dose of asbestos into the lung followed by measurements over periods of 2 weeks up to 2 years. Such experiments indicate a multi-term exponential clearance pattern typically predicting negligible retention 1 to 2 years after exposure. An example of such a curve is one which has been derived by MORGAN & HOLMES[5] at AERE HARWELL UK which is shown in Fig. 1. In this investigation the experimental animals (rats) inhaled fibres which had been activated by irradiation in the DIDO reactor and the clearance from the lungs was followed by measuring the decrease in radioactivity count. As can be seen in Fig.1.1 there was an immediate rapid clearance of fibres on cessation of exposure followed by a long period of more gradual clearance. This is shown more clearly if the data is plotted on arithmetic axes Fig. 1.2. If this curve is extrapolated to 365 days the retention as measured by the radioactivity count is reduced to 1%. After 2 years it is 0,1% of the original lung load.

Such curves are not compatible with what is found when the lungs of miners who have inhaled typical industrial concentrations of asbestos fibres over periods of 25 years or more and who have survived a further 20 years after exposure are examined. Under these conditions high concentrations of asbestos fibres may still be found in the lungs. Clearly the exponential clearance found in the animal experiments has not taken place in man at the rate predicted from these experiments.

Research workers at the Institute of Occupational Medicine, Edinburgh have recently proposed that a process of "sequestration" takes place when the lung clearance mechanism is grossly overloaded.[6,7] This theory suggests that excess fibres are stored in "compartments" of the lung

away from the main stream of clearance to allow the continued clearance of incoming fibres. The present work tends to support this theory. As a corollary to these investigations reference is made to parallel studies carried out at this centre in which the lungs of asbestos miners were examined. A major difficulty in this research was to establish the dose of asbestos received during the workers' life times. However it was possible to estimate this from the "Yellow Card Record" of each miner's service. This system of recording a miner's dust exposure in the asbestos mining industry was established in 1970 by the Cumulative Dust Exposure Records Committee (CUMDER)[8] of which the author was a member. Inspectors of the Air Quality Section of the Department of Mineral and Energy Affairs established mean fibre concentrations which could be expected during different operations on the mines. When used in conjunction with a man's record of service (Yellow Card) his cumulative exposure to asbestos fibres or fibre dose could be calculated. Prior to 1970 exposure or dose could not be estimated so closely.

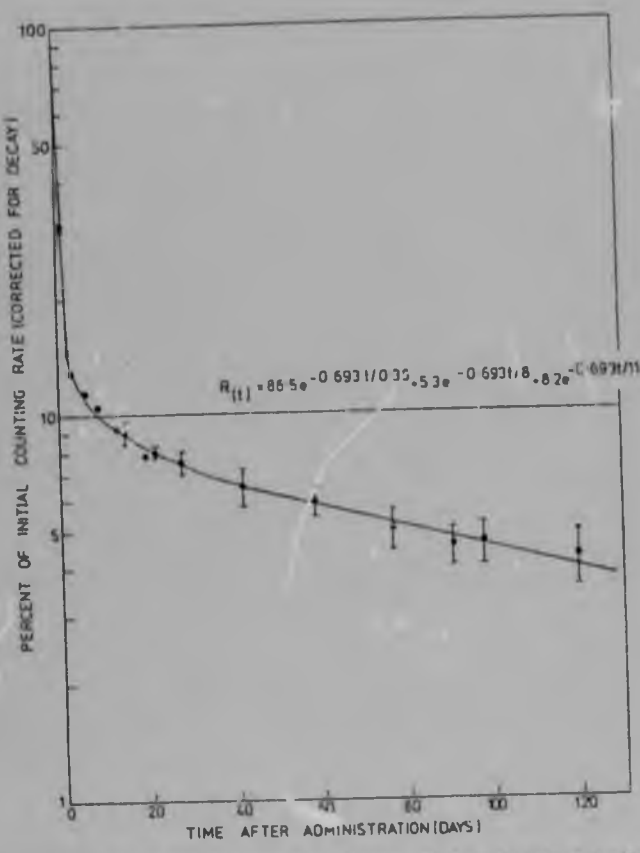
Because of the large variation in the estimated fibre dose received by different miners during their lives it was necessary to plot the ratio

$$\frac{\text{lung fibre load at death}}{\text{Estimated fibre dose in life}} \times 100$$

which is an index of lung clearance against the period of survival. If this index decreased with increasing period of survival it would indicate that fibre clearance was taking place. A slow but significant clearance was detected indicating a half life of 6 years for crocidolite asbestos. This is equivalent to a period of 36 years after cessation of exposure for 99% clearance. However the spread of the data was such that

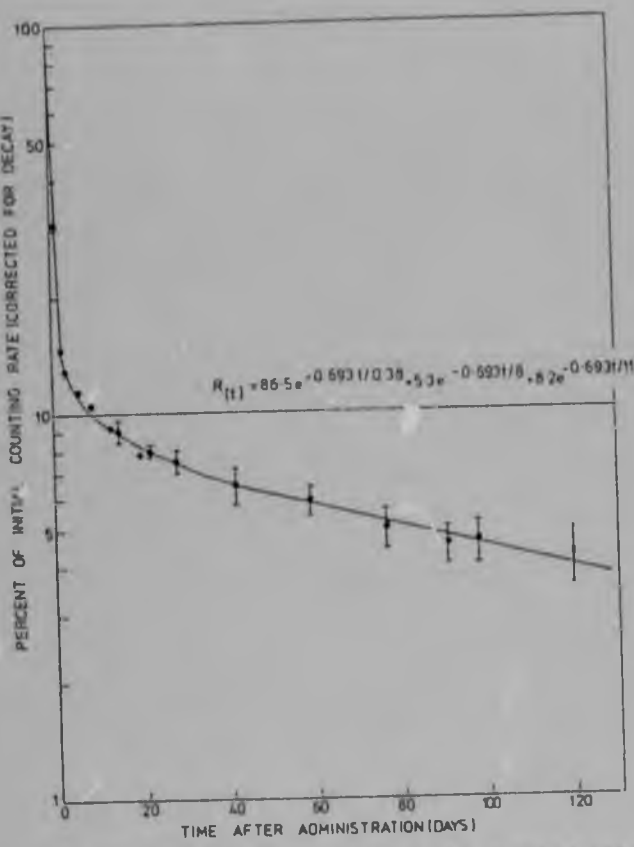
the 95% confidence limits of this figure were between 20 and 300 years indicating that many men would retain a high concentration of fibres in their lungs at death.

A graph derived from the examination of the lungs of miners and an estimation of their dust dosage during their working life is included as an appendix to the main study. (Appendix D)



Measurements of animal radioactivity (corrected for decay) at various times after inhalation exposure to synthetic fluoramphibole. Mean result for three animals expressed as a percentage of the counting rate measured immediately after exposure.

FIG 1.1

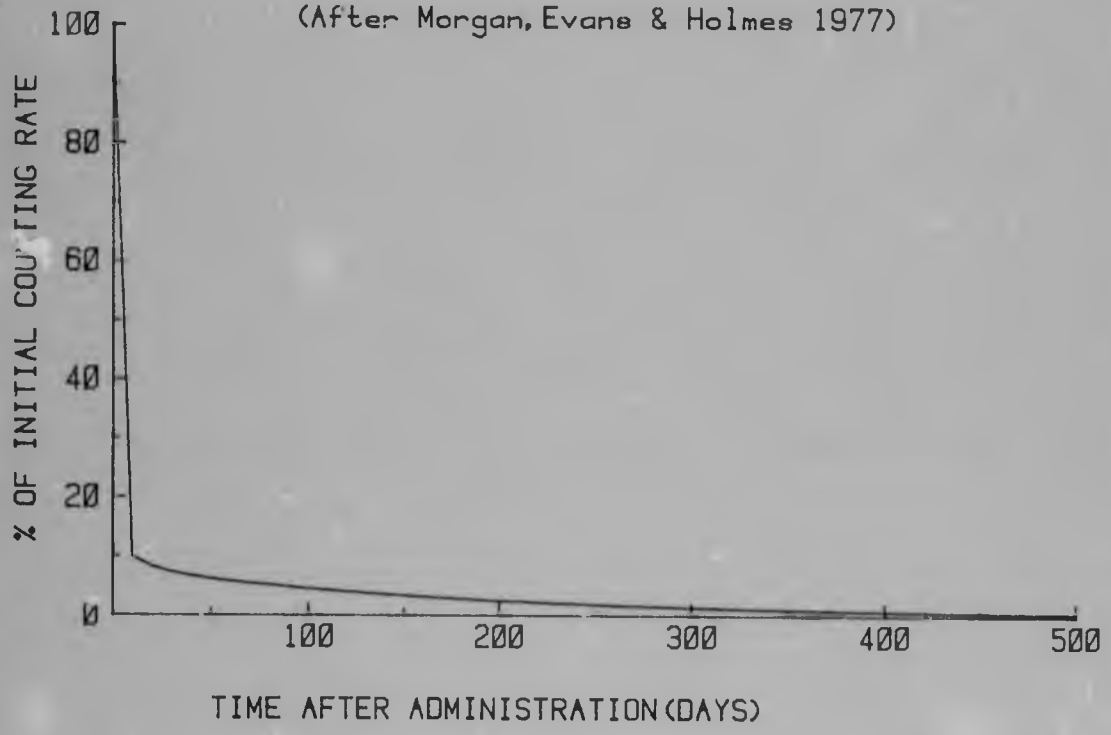


Measurements of animal radioactivity (corrected for decay) at various times after inhalation exposure to synthetic fluoramphibole. Mean result for three animals expressed as a percentage of the counting rate measured immediately after exposure.

FIG 1.1

LUNG CLEARANCE (RATS)

(After Morgan, Evans & Holmes 1977)



RDCR12/87

FIG 1.2

CHAPTER 2

REVIEW OF LITERATURE

Many studies have been made of retention and clearance of mineral fibres from the lung using animal models. This chapter reviews and comments on these published studies especially their relevance to the present investigation.

Among the first properly controlled studies of the effects of inhalation of asbestos using animal models were those carried out by Wagner[9] at the MRC Pneumoconiosis Unit at Penarth, UK. Though these studies were primarily concerned with the biological effects of inhaled asbestos, especially the induction of tumours, some reference is made to the relative retention of different types of fibres after different periods of exposure. In this experiment a large number of rats (1000) were used.

The main finding was that far less, of the order of 1/10th, of chrysotile is retained in the lung compared with amphibole asbestos. A second finding was that significantly less dust (70%) was retained by female than male animals. Although this is some of the earliest work done on asbestos inhalation and although measurement of fibre clearance was only a minor part of the investigation, it is one of the most realistic as far as the period of exposure and survival are concerned. Furthermore the large number of animals used places the findings on a firm statistical basis.

Another group actively engaged in research into lung retention and clearance of dust is that at the Institute of Occupational Medicine at Edinburgh, UK. In two related papers[10,11] Middleton described experiments in which rats were exposed to 3 different concentrations of UICC samples[12] of the 3 commercially important varieties of asbestos, amosite, crocidolite and chrysotile. Exposure was by inhalation over a 6 week period. This work is interesting in as much as 5 days were allowed after cessation of exposure for recently deposited asbestos to clear from the bronchial tract and major airways before the first animals were killed. This allowed a truer picture of the clearance from the deeper regions of the lung to be seen unobscured by fibres deposited just before the animal died. The results of this work showed that different types of asbestos are initially retained in different amounts and are cleared at different rates from the lung. In particular chrysotile is cleared more quickly than the amphiboles, amosite and crocidolite. It was hypothesized that low concentrations and light retentions are cleared proportionately faster than the heavy retentions due to high airborne concentrations.

The second paper of this pair[11] is largely devoted to the derivation of mathematical formulae to describe the clearance of fibres from the lung. For this purpose the lung was divided into 3 compartments each of which showed an exponential clearance curve. The curves overlapped and had differing half lives. An innovation in this series of experiments was 'Nocturnal' dust exposure. It was thought that the greater activity of rats at night might lead to a different pattern of retention and clearance but this was found not to be so.

At the end of this paper the important point is made that the clearance model used to describe the results of short term experiments is not applicable to long term inhalations. The latest contribution from I.O.M. Edinburgh has queried the validity of the exponential clearance pattern postulated by Middleton in as much as it does not seem to be applicable to the clearance of high dust doses. Instead the authors Bolton R.E. et al 1983[6] and Vincent J.H. et al 1985[7] propose an overload theory in which excess fibres are sequestrated in compartments of the lung away from the main stream of clearance. This theory is more in accordance with our own experience with the lungs of miners who have had high dust exposure and long periods of post exposure survival.

In 1977 two very experienced researchers in the field of lung clearance, A. Morgan and A. Holmes of the Atomic Energy Research Establishment (AERE) Harwell U.K. reported on the deposition and clearance of inhaled fibrous minerals. Radioactive tracer techniques were used to monitor the initial deposition and subsequent clearance of fibres[5]. Rats were exposed in a "nose only" inhalation chamber for 30 mins to different types of fibre which had been suitably activated. No reference is made to the actual dust concentration but this may be inferred from another paper[12] to be about 20 mg/m^3.

The lung clearance, which was monitored by measurement of the radioactivity, was followed for about 100 days. Separate measurements were made of clearance in the upper and lower respiratory tracts, half lives being calculated for each animal. These varied between 46-76 days. The clearance was also illustrated by the use of autoradiographs of whole lung sections.

Later work by the same authors[13] using activated anthophyllite asbestos demonstrated that fibres less than 5 µm long are removed more rapidly than longer fibres. In this experiment the fibres were introduced into the lung by short term inhalation and the period of survival after inhalation was extended to 205 days. The fibres collected by the scavenging macrophages and those retained in the tissue were evaluated separately.

The criticism of this work is that the period of exposure was too short to reveal the whole picture. Another criticism is that only one type of fibre and that a fibre infrequently used commercially was investigated.

In their latest paper A. Morgan and A. Holmes[14], describe and compare the clearance of glass fibres of different length and diameter from the lungs of rats. As a secondary objective they investigated the effect on the fibres of long term (>1yr) residence in the lung. Because rats cannot inhale coarse glass fibre (diameter 1,5-3,0 µm) normally, the fibres were placed in the lungs by intratracheal instillation. Photo micrographs of the fibres before and after one year's residence in the lung show how the fibres, especially the long ones, have been dissolved or etched increasingly towards the ends reducing them from parallel sided cylinders to spicules pointed at each end. With regard to the primary objective, the comparison of the clearance of fibres of different lengths the investigation found that though 80% of the fibres up to 10 µm length were cleared after a year there was virtually no clearance of fibres greater than 30 µm long. The method used in this investigation was to irradiate the glass fibres in the DIDO reactor to produce the radionuclide ^{24}Na. The irradiation was used to check the

initial instillation of the fibres and later to investigate their solubility in the reagent used to destroy the tissue during the fibre extraction process. The clearance of the fibres was monitored by normal lung digestion and collection of the fibres on filter paper. Counting of fibres was done under a light microscope using the conventional phase contrast method. (See Chapter 7)

As was to be expected from these workers this was a first class piece of work but it is to be regretted that no investigations were carried out with fibres intermediate in length between 10 μm and 30 μm. Furthermore the method of intratracheal instillation is a very harsh and traumatic method of introducing fibres into the lung and one that has been shown to reduce the survival time of the animals[9]. It has also been shown[15] that the distribution of dust in the lungs after intratracheal instillation is very different from that after prolonged inhalation. This can influence profoundly the rate of clearance especially in the early stages.

Despite the high technology employed in these investigations the conditions do not truly relate to those in the real world. Both inhalation and residence periods are too short for the effects due to lung disease to be included. The authors do recognise the latter shortcoming and consider that under actual conditions where the lungs may become diseased, clearance would be slower than their experiments indicate.

In a recent paper[16] Roggli described how rats were exposed to a dust cloud of chrysotile asbestos at a respirable concentration of 15 mg/m^3

for 1 hour in a "nose only" exposure chamber. Some animals were killed immediately and others at 1, 8, 14 and 31 days after exposure. Inhaled fibres in the lungs were estimated by digestion in sodium hypochlorite solution to destroy the lung tissue and counted under a scanning electron microscope. From this examination the number of fibres per gram of lung tissue was calculated. The total mass of fibres retained in the lung was then estimated from this figure. The mass of fibre inhaled during the inhalation period was calculated from the respiratory rate x the tidal volume of the rat (assumed to be 0,1 ℓpm) x time of exposure. This procedure successfully demonstrated a progressive decrease in fibre load and fibre diameter and an increase in mean fibre length over the 31 day survival period. This appeared to be a well thought out and well conducted experiment but suffered from the limitation that the exposure period and survival time were too short to be representative of real life conditions where exposure may extend over years and lung clearance may deteriorate due to smoking or disease. Also it was not possible to take into account reactions which may take place between the lung and the fibres such as leaching or breakage.

This review of the literature has shown that [illegible]ircumstances under which much of the experimentation on fibre clearance has taken place, have been very artificial and divorced from the realities of industrial exposure. In particular exposure times have been too short and the animals have not been allowed to survive long enough. Also it is questionable whether the results obtained from such small animals as rats can be extrapolated directly to human beings. I feel that the

material available in this Centre from baboons which have been exposed for long period i.e. 2-4 years and have been allowed to survive for a similar period will more closely simulate the conditions under which human exposure occurs.

CHAPTER 3

MATERIALS USED

In this chapter the materials use in the investigation are described in 2 sections.

3.1 Mineralogical characteristics of the fibres

3.2 Physical dimensions of the fibres

3.1 Mineralogical characteristics

Two types of material were used namely three varieties of asbestos which are naturally occurring mineral fibres, and glass fibre (1 variety) which is a member of the group usually referred to as man made mineral fibres (MMMF).

3.1.1 Asbestos

There are two types of fibrous minerals which are commonly called asbestos, namely

3.1.1.1 Amphiboles

3.1.1.2 Serpentine

3.1.1.1 The amphiboles are a large group of rock forming minerals, some of which are in the form of fibres and are called asbestos. The amphibole asbestos, in this case the varieties, amosite and crocidolite, are stiff straight needles, the needles of crocidolite being finer than those of amosite[22]. See Figs 3.1.1.1.1 and

3.1.1.1.2. Crystallographically both consist of parallel chains of SiO_4'''' tetrahedra[23], (the fundamental building blocks of rock forming minerals) linked together by different metallic ions. Although the composition of asbestos varies slightly from sample to sample the generally accepted formula for crocidolite is $Na_2Fe_3\ Fe_2(Si_4O_{11})_2OH_2$. The $(Si_4O_{11})''''$ group being a double chain of SiO_4'''' tetrahedra joined at 2 apices. Similarly the formula for amosite is $(Fe, Mg)_7\ (Si_4O_{11})_2\ OH_2$. A simplified diagram showing the relationship between the commoner fibrous amphiboles is shown in Fig. 3.1.1.1. Amphibole asbestos which is very durable and has a high tensile strength is used mainly for reinforcing, as in asbestos cement products or for high temperature insulation in power houses.

3.1.1.2 Serpentine

Serpentine asbestos or chrysotile (Fig. 3.1.1.2) is actually a sheet mineral like talc but the sheet is bent into a tube or "Swiss roll" structure because of a mismatch between the lattices of the two components which form it. The diameter of the tube is of the order of 0,1µm. The formula for chrysotile is $Mg_3\ (Si_2O_5).(OH)$ which is similar to that for talc. The Si_2O_5'' group being a sheet configuration of $(SiO_4)''''$ tetrahedra joined at 3 apices.

Chrysotile fibres are much softer than the amphibole fibres and can be opened out into a soft fluffy material suitable for spinning and weaving.

The asbestos fibres used in the experiments were drawn from a stock of finely ground asbestos samples specially prepared for animal experimentation. They are generally referred to as the UICC (Union Internationale Contre le Cancer) samples. These samples were prepared in Johannesburg by the Occupational Hygiene Department in 1966 following a decision taken at the UICC Conference on the Biological Effects of Asbestos held in New York in 1964[18]. The purpose of the samples was to provide a source of uniform material which could be used by all investigators involved in research into the biological effects of asbestos.

A full description of the preparation of the samples has been published[19] and their physical and chemical characteristics have been studied and documented[20,21].

The main stock of samples has been held in and distributed world wide from Johannesburg for the past 20 years.

3.2.2 Glass Fibre

Glass fibre is spun from different types of glass and is generally thicker than asbestos fibres. Its general

appearance is shown in Fig. 3.1.2. There are many different types of glass fibre just as there are different types of asbestos. The glass fibre used in this investigation was obtained from Johns Manville Asbestos Mining Co. Canada. It is a blend of the two finest products available, referred to as TIMA C104 and C106. TIMA is an acronym for Thermal Insulation Manufacturer's Association.

3.2 Physical Dimensions

The dimensions of the four fibres (in microns, μm) are presented in Tables 3.1. and 3.2 which show the size distribution by length, and by diameter. The full size distributions by length and diameter together are included as Appendix A. These tables include histograms to assist in the rapid assimilation of the data. The dimensions of the fibres were fed directly into the computer from electron micrographs similar to Fig. 3.1.1.1 by use of a digitizing platen. The computer performed the necessary sorting and classification by use of specially written programme.

Table 3.1 Fibre length distribution

Fibre Type	< 10μm	> 10μm	n
Glass fibre	41,4%	58,6%	198
Crocidolite	96,5%	3,5%	199
Amosite	90%	10%	199
Chrysotile	83,9%	16,1%	168

appearance is shown in Fig. 3.1.2. There are many different types of glass fibre just as there are different types of asbestos. The glass fibre used in this investigation was obtained from Johns Manville Asbestos Mining Co. Canada. It is a blend of the two finest products available, referred to as TIMA C104 and C106. TIMA is an acronym for Thermal Insulation Manufacturer's Association.

3.2 Physical Dimensions

The dimensions of the four fibres (in microns, μm) are presented in Tables 3.1. and 3.2 which show the size distribution by length, and by diameter. The full size distributions by length and diameter together are included as Appendix A. These tables include histograms to assist in the rapid assimilation of the data. The dimensions of the fibres were fed directly into the computer from electron micrographs similar to Fig. 3.1.1.1 by use of a digitizing platen. The computer performed the necessary sorting and classification by use of specially written programme.

Table 3.1 Fibre length distribution

Fibre Type	<10μm	>10μm	n
Glass fibre	41,4%	58,6%	198
Crocidolite	96,5%	3,5%	199
Amosite	90%	10%	199
Chrysotile	83,9%	16,1%	168

These figures refer to the fibres in the dust cloud. After residence in the lung the % of fibres >10μm may be very different. Glass fibres in particular have been shown to break up rapidly and short fibres are cleared from the lung faster than long fibres. Fibres longer than 10μm are generally considered to be the most biologically active.

The most important dimension of a fibre is generally considered to be its diameter. Fibres less than 0,32 μm in diameter are not visible under a light microscope fitted with the x40 air objective used for fibre counting and are generally referred to as submicroscopic fibres.

Table 3.2 Fibre diameter distribution

Fibre Type	<0,32μm	>0,32μm	n
Glass fibre	30,3	69,7	198
Crocidolite	44,2	55,8	199
Amosite	27,1	72,9	199
Chrysotile	25,6	74,4	168

It should be noted that the experimental glass fibre is finer than the commercial product normally used though a certain proportion of submicroscopic fibre has been found in the atmosphere of a glass fibre factory.

It should also be noted that although the chrysotile fibres appear to be the coarsest of the groups they rapidly disintegrate into fine fibrils in the lung.

The interpretation of the two dimensional data is not so straightforward. This part of the computer programme was written so that rapid comparisons could be made of size distributions of fibres after different periods of residence in the lung. It was considered to be outside the scope of this dissertation to investigate these changes

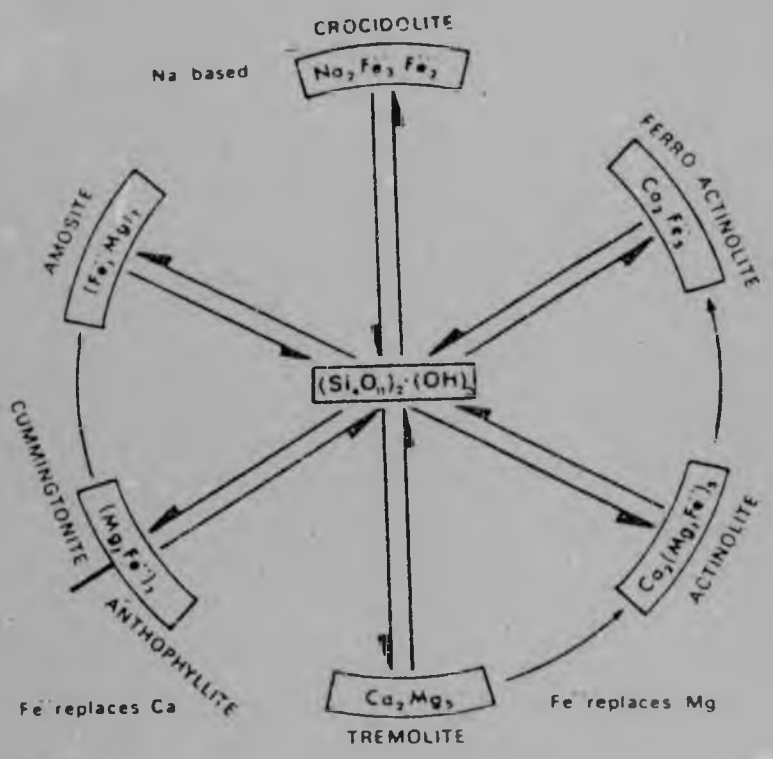


FIG 3.1.1.1 Relationship between the commoner fibrous amphiboles.

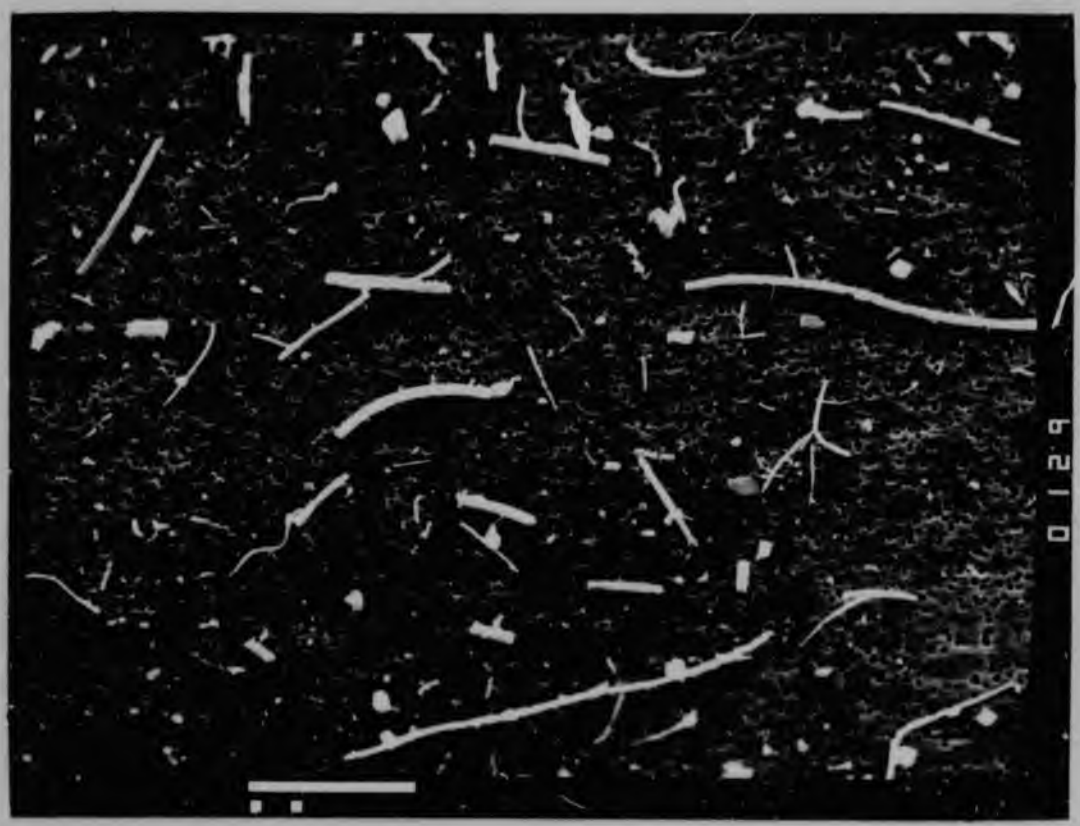


FIG 3.1.2 Glass fibre (x2500)

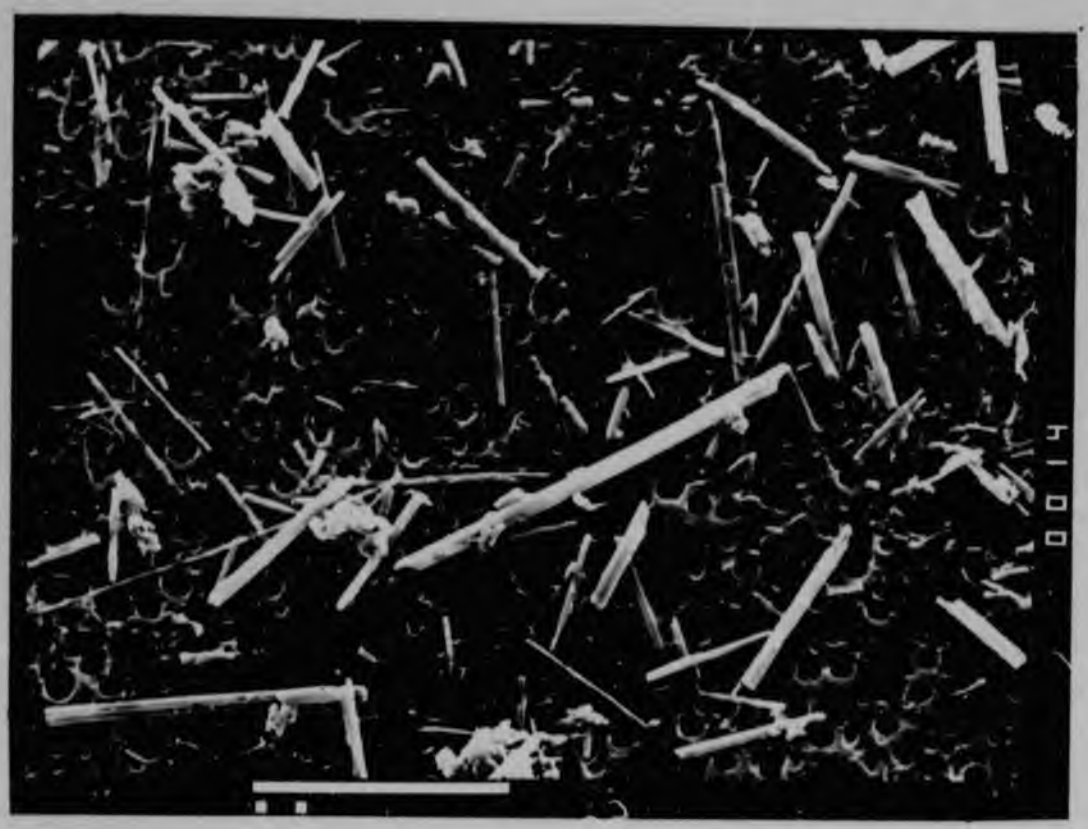


FIG 3.1.1.1.1 Crocidolite fibres (x4000)

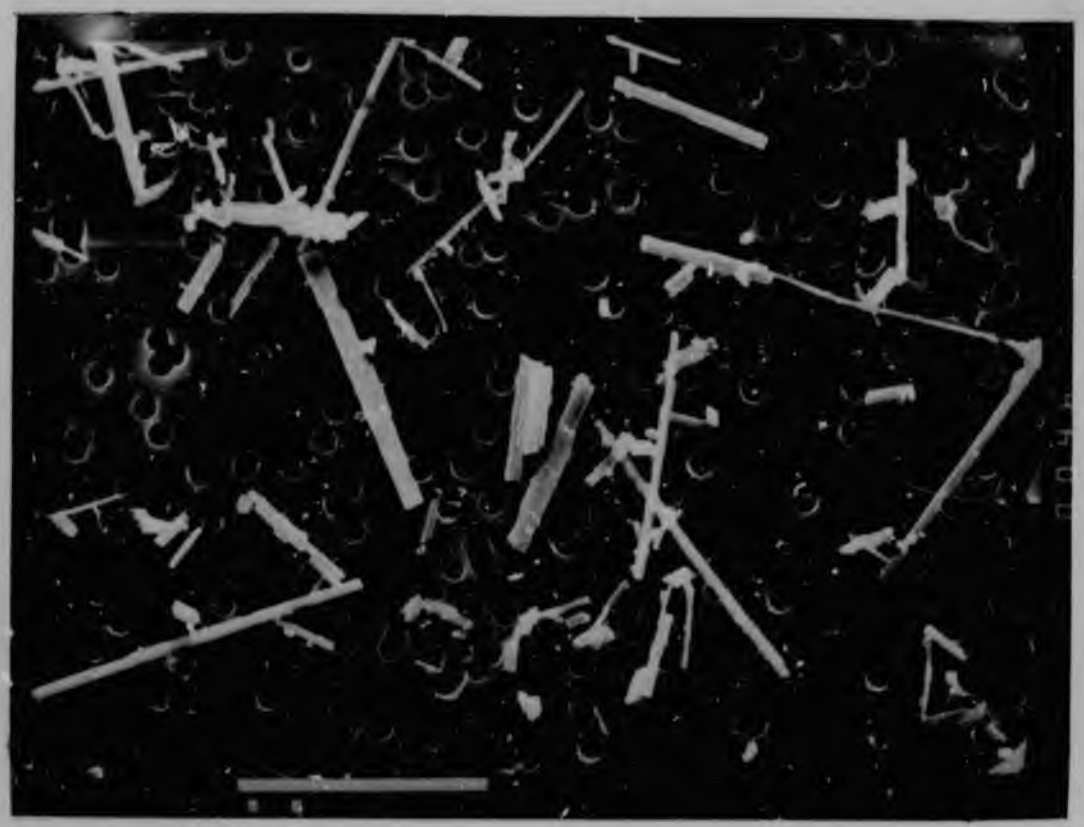


FIG 3.1.1.1.2 Amosite fibres (x4000)

FIG 3.1.1.2 Chrysotile fibres (x2500)

CHAPTER 4

DUST INHALATION

In this chapter the inhalation facility in which the animals, in this case indigenous Chacma baboons (Papio Ursinus) inhaled the different dusts is described and details of their exposure and survival times are given.

The dust concentrations are expressed in fibres per cubic centimeter of air (fpcc). This method of denoting concentrations is fully discussed in Chapter 7. The full statistics of the dust exposure are addressed in Chapter 11.

4.1 Inhalation Facility

The layout of the inhalation facility is shown in Fig. 4.1. It consists of a corridor pressurized to 250 Pa (1" water gauge) by means of an axial flow mine ventilation fan. On either side of the corridor are 7 chambers each approximately 3mx3mx3m of which 10 are available for inhalation experiments. Four of these rooms have been modified to accommodate separate exposure chambers for smaller animals. These were not used for the baboon exposures. The remaining four are used for dust preparation, cold storage of food, wash up, etc.

In the corridor outside each chamber is a compressed air apparatus to disperse dust continuously into the chamber. Details of this dust dispersion system have been published[25]. The aim of the dust

dispersion system is to maintain the dust concentration in the rooms as constant as possible over the whole of the 7 hour inhalation period.

On the outside wall of the building and connected to each chamber are flannel bag air filters to remove the dust from the air passing through the chambers before it is returned to the atmosphere. Dusting takes place 5 days a week except on public holidays. The experimental animals are caged within the chambers in numbers according to their size. In the case of baboons 12 are normally caged singly in separate cages. Most of the inhalation experiments last two to three years though this period may be reduced or extended as required. During the inhalation period each animal is inspected daily and any that are sick are removed for treatment. A complete record is kept of the exposure of each individual animal.

Dust concentrations are monitored daily or as frequently as possible using instruments appropriate to the measurement required (see Chapter 5). This may be the daily average total dust concentration measured in milligrams of dust per cubic metre of air (mg/m^3), average respirable dust concentration (mg/m^3) or number of fibres per cubic centimetre of air (fpcc). Special samples may also be taken for electron microscopy which are needed to determine fibre size (length or diameter) distribution. For special experiments where changes in concentration are called for during the exposure, continuous recording instruments are available.

The most commonly used indicator of concentration is the daily average respirable dust concentration (mg/m^3). In the case of asbestos or glass fibre dusts these samples are supplemented by fibre counts. Because of the high concentration of fibres in the chambers it is not possible to take extended samples for fibre counting. These samples are usually taken over a period of 30-60 seconds randomly throughout the day. As the evaluation of fibre count samples is a lengthy procedure these samples cannot be taken as often as gravimetric samples which only require weighing.

The gravimetric samples are used to control the dust concentration in the chambers but the parameter of biological interest is the number of fibres per cubic centimeter so it is necessary to take both types of sample.

4.2 Dust exposure and survival after exposure

Details of the exposure and the survival time of all the animals involved in the various experiments are best explained by setting them out in chart form. This has been done in Figs. 4.2.1-4.2.4.

4.3 Dusting Conditions

The dusting conditions in the different exposure chambers will now be considered in greater detail.

In all cases the target average concentration over the whole of the dust period was 1000 fpcc. However it should be noted that the fibre count is subject to variations due to the counter[45,46] This factor is discussed in more detail in Chapter 7.

4.3.1 Glass Fibre

From Fig. 4.3.1 it can be seen that the concentrations at the beginning of the exposure period exceeded the 1000 fpcc target. It was therefore necessary to bring the concentration down gradually. This was done by calculating a running average concentration based on past exposure and adjusting the dust dispersers down each day until a figure as near as possible to 1000 fpcc was reached. Initially about 45 grams of dust were dispersed into the chamber per day but this was gradually reduced to 30g/day.

4.3.2 Crocidolite

Fig. 4.3.2 depicts the course of the crocidolite exposure. In this chamber the same situation as in the glass fibre chamber (See 4.3.1) prevailed, i.e. the initial concentration was too high. However in this case, the pathologist concerned considered that the health of the animals was being acutely affected and that the concentration must be brought down rapidly. This exposure should therefore be considered in two parts; an initial period in which the concentration was brought down from more than 4000 fpcc to 1000 fpcc and a second part in which the average concentration was maintained at 1000 fpcc. This was achieved by reducing the amount of dust dispersed from 70g/day to 35g/day. The fibre concentration in the chambers is not linearly related to the amount of dust dispersed but rises more rapidly.

4.3.3 Amosite

The course of the amosite exposure is presented in Fig. 4.3.3. Apart from an inexplicable peak about the middle of the exposure period in which the concentration rose to more than 1000 fpcc, it oscillated randomly about the 1000 fpcc target throughout the exposure period.

4.3.4 Chrysotile

Chrysotile is the most difficult of all the fibres tc disperse and to sample. The exposure record is set out in Fig. 4.3.4. The problem in th's chamber was the reverse of that in the others. In this case it was necessary to try to increase the amount of dust dispersed per day. However, this apparently induced a high mortality among the animals so the concentration had to be reduced again.

Table 4.1 Summary of Fibre Exposure C. 'itions

Fibre Type	Period (Mnths)	Conc. (fpcc)	S.D. (fpcc)
Glass fibre	34	1085	421
Crocidolite	35	1082	631
Amosite	49	1111	517
Chrysotile	49	735	287

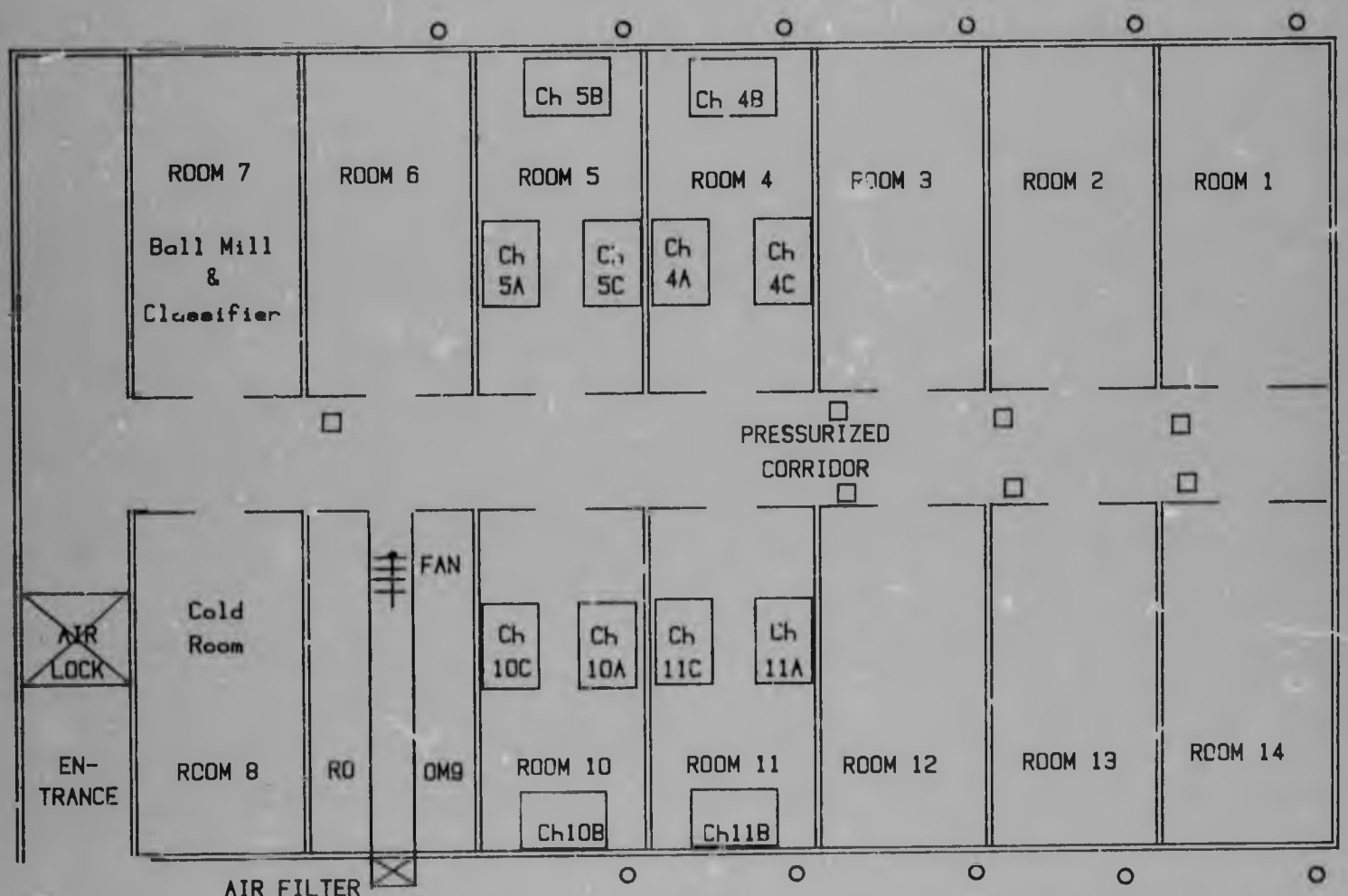


FIG 4.1 Animal dusting facility.

REGR101287

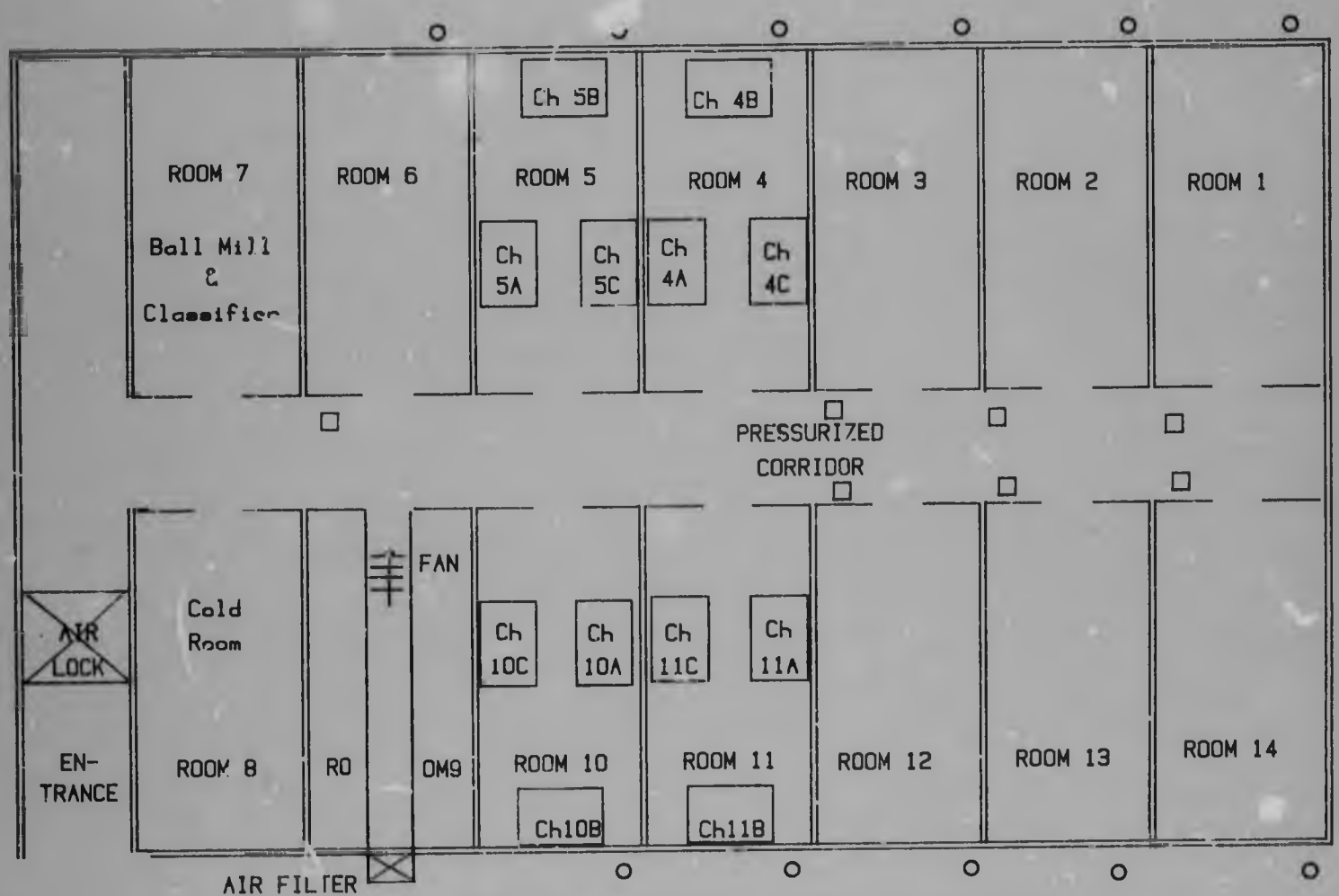


FIG 4.1 Animal dusting facility.

REGR101287

HISTORY OF BABOONS EXPOSED TO FIBRES

GLASS FIBRE 30-8-77 TO 9-7-80

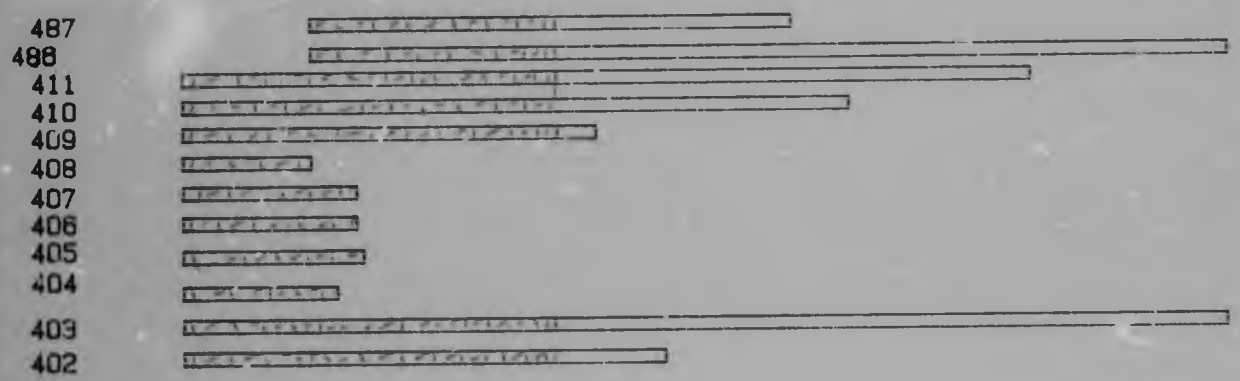


FIG 4.2.1

CROCIDOLITE 9-8-76 TO 30-6-79

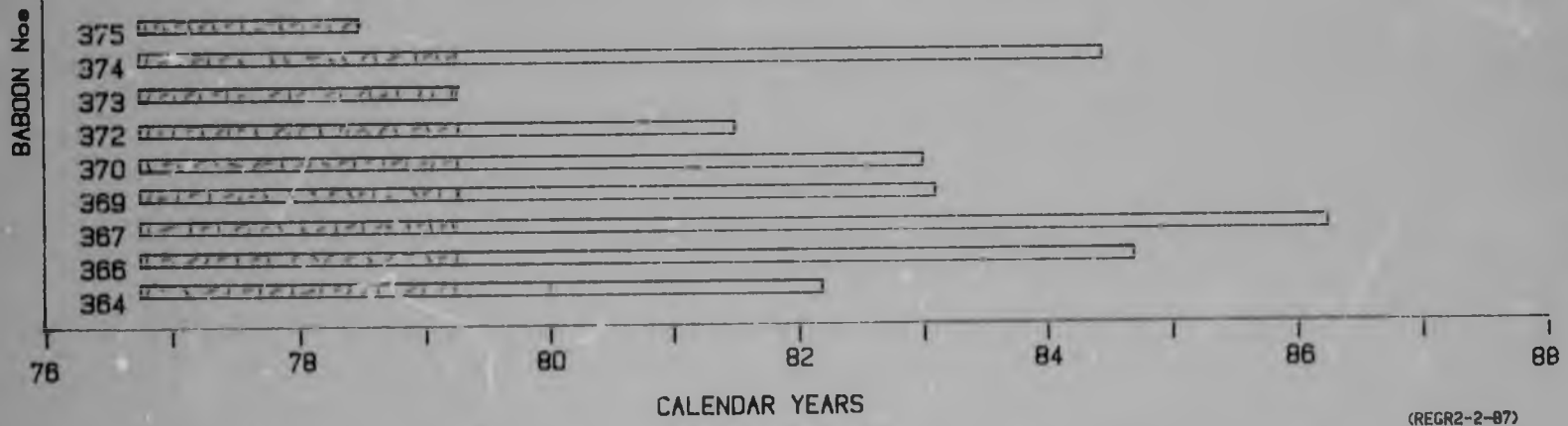


FIG 4.2.2

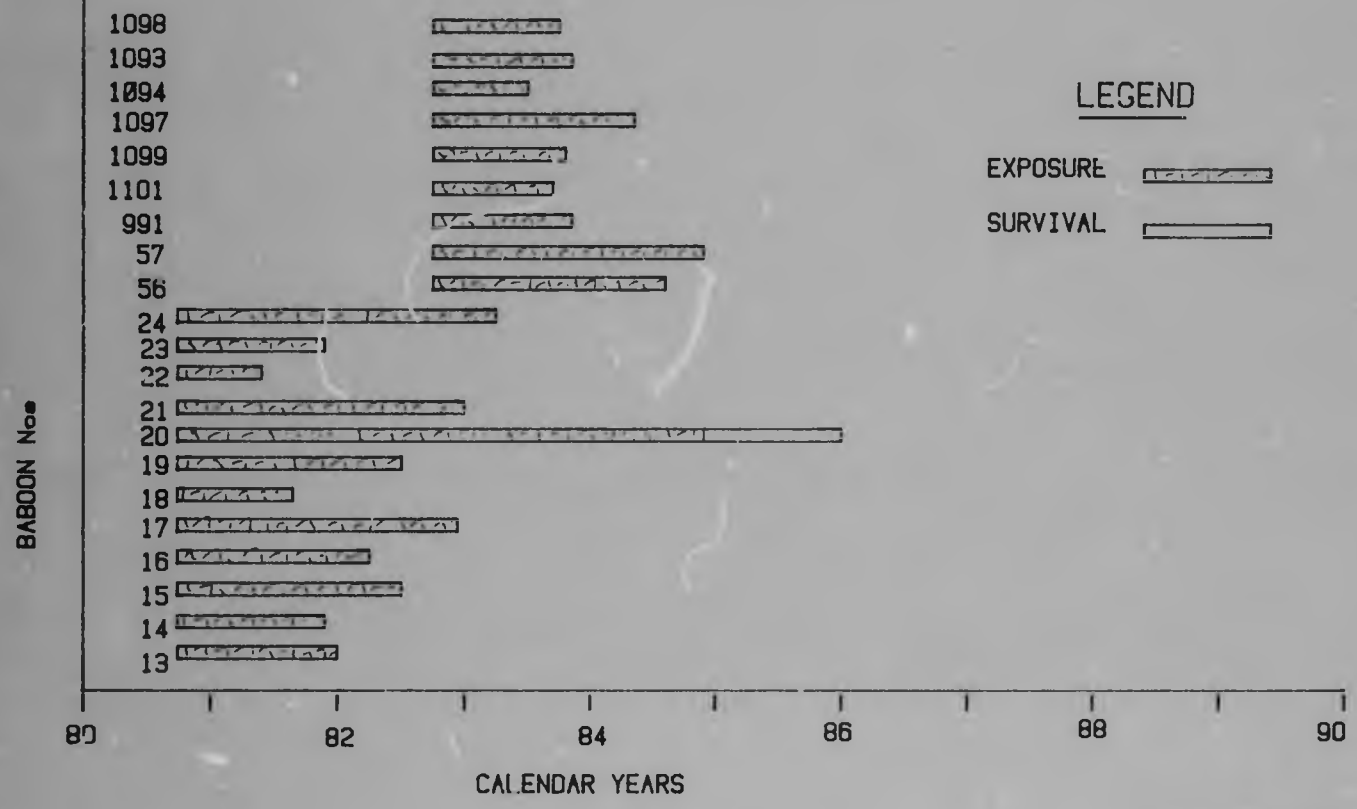
HISTORY OF BABOONS EXPOSED TO FIBRES (Cont)

AMOSITE 15-9-80 TO 20-10-84

FIG 4.2.3

HISTORY OF BABOONS EXPOSED TO FIBRES (Cont)

CHRYSOTILE 15-9-80 TO 19-10-84



(REGR3-2-87)

FIG 4.2.4

FIBRE EXPOSURES OF BABOONS

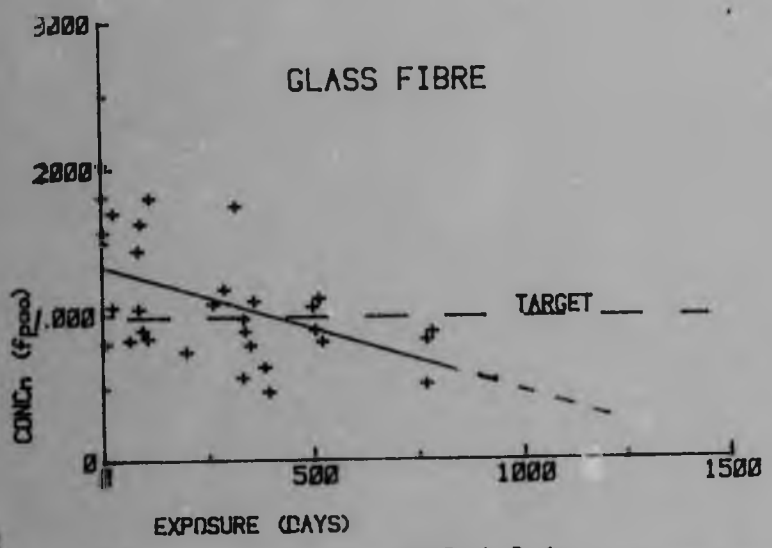


FIG 4.3.1

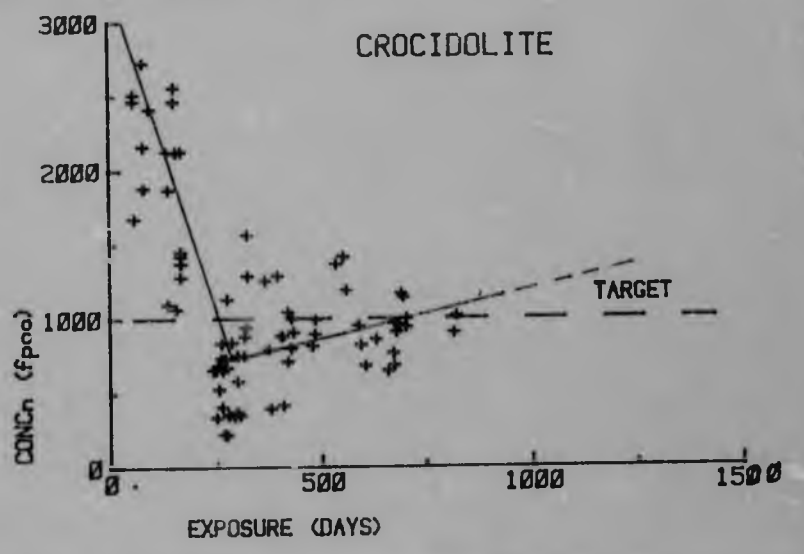


FIG 4.3.2

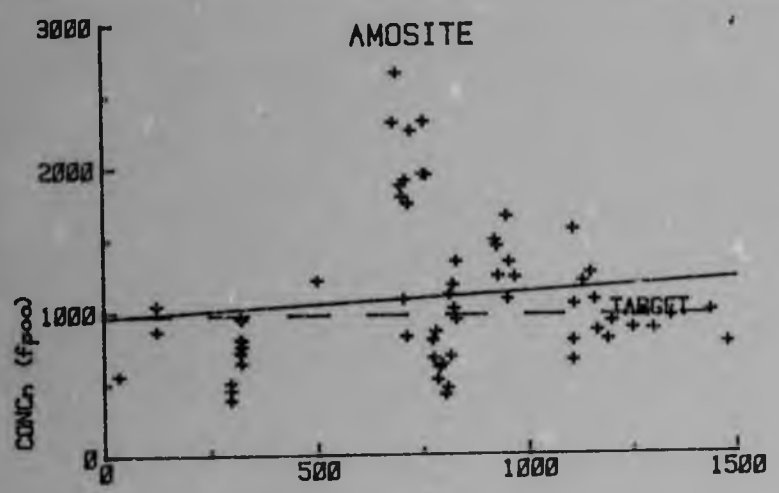


FIG 4.3.3

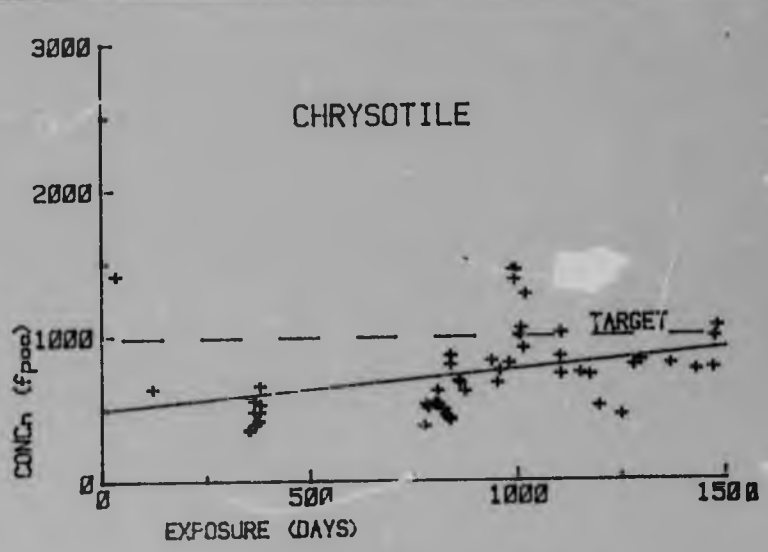


FIG 4.3.4

CHAPTER 5

DUST SAMPLING INSTRUMENTS

There are many different types of instrument designed to measure the dust content of the air[26]. They are referred to as dust samplers and operate on many different principles.

For convenience these dust samplers may be sub-divided as follows:-

5.1 Gravimetric samplers

- 5.1.1 Total dust samplers
- 5.1.2 Respirable dust samplers

5.2 Samplers for fibre counting

- 5.2.1 Membrane filter samplers
- 5.2.2 Gold coated Nuclepore samplers

5.3 Direct reading samplers

- 5.3.1 Gravimetric read out
- 5.3.2 Fibre count read out

5.1 Gravimetric Samplers

Gravimetric samplers are samplers which collect weighable amounts of dust from the air. Since the concentration of dust in the air is usually measured in milligrams or fractions of milligrams of dust per cubic metre of air and it is not practicable to weigh masses less than 0,2 mg accurately on a conventional laboratory balance these instruments must have a high sampling rate, of the

order litres per minute, in order to collect weighable amounts of dust. Most commonly these instruments suck a known volume of air through a special air sampling filter which is weighed before and after sampling to determire the weight of dust collected.

The volume of air sampled by the instrument is determined by multiplying the flow rate in litres/minute by the sampling time in minutes; therefore the flow meter on the instrument must be accurately calibrated before sampling. This is best carried out with a bubble flowmeter in which the actual volume of air sampled can be measured over a fixed time but it may be done using an accurate master flow meter. It is important to measure the sampling rate with the filter in place. Different types of filters are used for different purposes. A good general purpose filter for gravimetric sampling is glass fibre. However glass fibre filter papers are not suitable for samples on which it is intended to make a quartz determination.

Gravimetric sampling instruments may be divided into two classes.

5.1.1 Total dust samplers

5.1.2 Respirable dust samplers

5.1.1 Total dust samplers

These instruments collect a sample of airborne dust particles regardless of size. They usually consist of an electric motor, which may be driven either by batteries or mains power, a suction air pump, either reciprocating or rotary vane type, and a filter holder suitably

connected to the pump. The connection may be a rigid pipe or a flexible tube. An air flow meter also is usually included either just before the filter holder or on the exhaust. The flowmeter is generally of the rotameter type in which a glass or metal ball rests at the bottom of a tapered glass or plastic tube and is carried up the tube by the air flow to a height proportional to the rate of flow. The tube may be calibrated in flow units directly or in fractions of the full flow rate.

5.1.2 Respirable dust sampler

In many cases the harmful effect of a dust is not proportional to the total dust concentration in the air but only to the concentration of the fine or 'respirable' fraction. In these cases a small cyclone is connected to the filter holder in front of the collecting filter, its purpose being to remove the coarse particles before they can reach the filter: in this way only the fine particles are collected. In other respects the respirable dust sampler is similar to the total dust sampler. The respirable dust concentration is typically a third or less of the total dust concentration but in the special finely ground dusts prepared for this investigation the proportion of respirable dust was 75-85%.

Both types of sampler are available in different sizes from large heavy instruments sampling large volumes of air to small samplers which may be carried on the person. The larger instruments are used to collect dust samples from the general background air while the smaller portable instruments measure the dust actually created by the wearer as he goes about his work.

A typical portable, or personal sampler is shown in Fig. 5.1.2. It is shown connected to an open filter for collecting a total dust sample. On the left is one of the cyclones used in respirable sampling and on the right is a filter holder with a cowl used for asbestos sampling.

5.2 Samplers for fibre counting

The toxic parameter of airborne fibrous minerals such as asbestos or man made mineral fibres (MMMF) is generally considered to be proportional to the number of fibres falling within a specified range of length and diameter which occur in a cubic centimetre of air. This matter is discussed in more detail in Chapter 7. The only method to evaluate the toxicity of clouds of such minerals is to collect samples from the air and count the number of fibres within the specified range.

Two techniques are employed to do this

1. Collection on membrane filter and counting under a light microscope. (See Chapter 7)

2. Collection on Nuclepore filters and evaluation under an electron microscope. (See Chapter 8)

5.2.1 Membrane filter samplers

The principle of this method is straight forward. Samples are collected on special cellulose acetate/cellulose nitrate filter paper which can be made as transparent as glass by suitable clearing agents[35,36]. After clearing, the fibres on the paper are counted under a light microscope.

From the fibre count and the volume of air sampled the airborne concentration expressed as fibres per cubic centimetre of air (fpcc) can be calculated. However in practice large variations in the value of the concentration as calculated by different counters occur[46,47]. This has lead to a strict procedure, laid down by the Asbestos International Association (AIA)[35] which must be observed when making asbestos counts. The purpose of the procedure is to eliminate as many of the sources of variation as possible.

The visual acuity of the microscopist is an important factor in counting as the diameters of the fibres decrease continuously from what is clearly visible under the light microscope to less than the wavelength of light. This means that fibres become fainter and fainter and there is no clear cut off. Work carried out in the

electron microscope department in the NCOH in which the same field of fibres was examined by light and scanning electron microscopy showed the finest fibre that could reliably resolved by the standard light microscope was 0,32µm. This work is discussed more fully in Chapter 7.

5.2.2 Gold coated Nuclepore filters

In order to count those fibres in the air whose diameters are less than the wavelength of light it is necessary to resort to the techniques of electron microscopy. For the highest accuracy the transmission electron microscope (T.E.M.) should be used but for routine work the scanning electron microscope (S.E.M.) is adequate.

Samples are collected on a smooth surfaced Nuclepore[27] filter which has been coated with Au/Pd to make it conducting. Unless this is done the sample rapidly charges up and the image disappears. Counting may be done on the S.E.M. but it is very much more laborious than on the light microscope. A specimen of gold coated Nuclepore is included as Fig. 5.2.2.

5.3 Direct reading Samplers

The most commonly used principle in direct reading instruments is light scattering by dust particles. In some cases it has been found advantageous to use infra red radiation instead of visible light.

5.3.1. Direct reading gravimetric instruments

In order to measure and control the dust concentration in the chambers in which the animals inhaled the fibrous dust an aerosol photometer was used[26]. The appearance and principle of this instrument are shown in Fig. 5.3.1.1.

The dusty air to be sampled is drawn down the intake tube at 17,5 litres/minute and sheathed in clean filtered air.

At a point on the central axis of the instrument there is a transverse hole in the in the tube on which the light from a high intensity bulb is focused. On the other side of the hole and protected from the direct beam is a photomultiplier tube (PMT) which measures the intensity of the light scattered from the thread of dust being drawn into the instrument. By suitable electronics and calibration the response of the PMT may be adjusted so that it is proportional to the concentration or log of the concentration of the dust being sampled. The instrument used in our experiments had a log response.

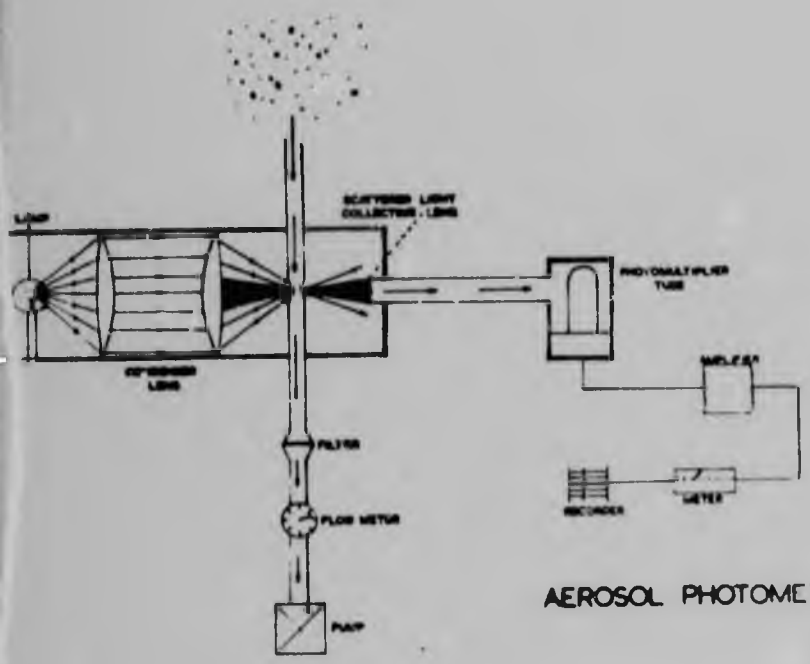
As an additional check on the instrument readings the dust sampled may be collected on a filter after it has passed through the light scattering portion of the instrument and the mean concentration over the sampling period determined and compared with the chart record.

5.3.2 Direct reading particle counters

An alternative method of estimating dust concentration is to draw the air through the instrument more slowly and illuminate each dust particle as it enters a very small ($1mm^3$) sensing zone. The flash from each particle is then counted electronically.

An elaboration of this principle is to set each particle spinning as it passes through the sensing zone and only count those particles with a pronounced flicker. The flickering particles are presumed to be elongated particles or fibres. By calibration with suitable fibrous aerosols the sensitivity of the instrument may be adjusted so only that fibres falling in the prescribed range are counted. Such an instrument is referred to as fibrous aerosol monitor (FAM)[48]. The FAM is illustrated in Fig. 5.3.2.

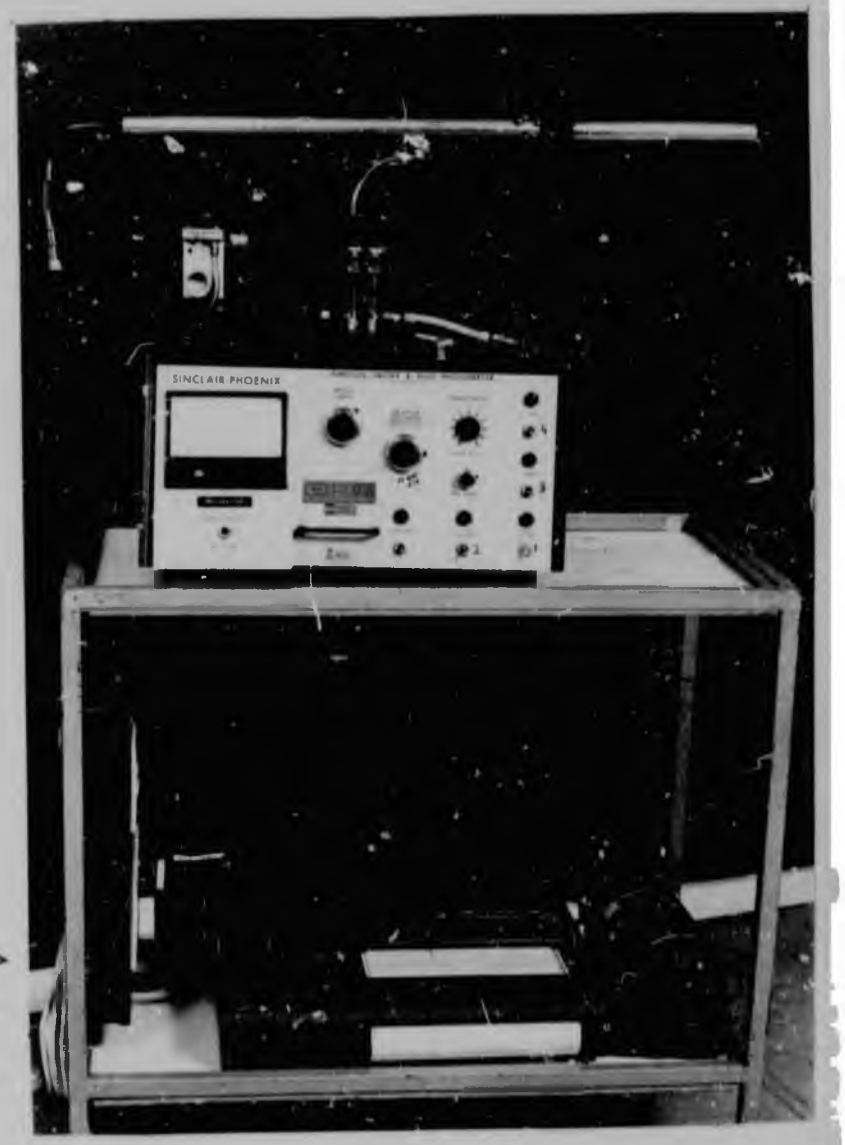
FIG. 5.2.2 GOLD COATED NUCLEPORE FILTER ON WHICH FIBRES ARE COLLECTED FOR SCANNING ELECTRON MICROSCOPY



AEROSOL PHOTOMETER

Principle

Appearance --->



AEROSOL PHOTOMETER
FIG 5.3.1.1

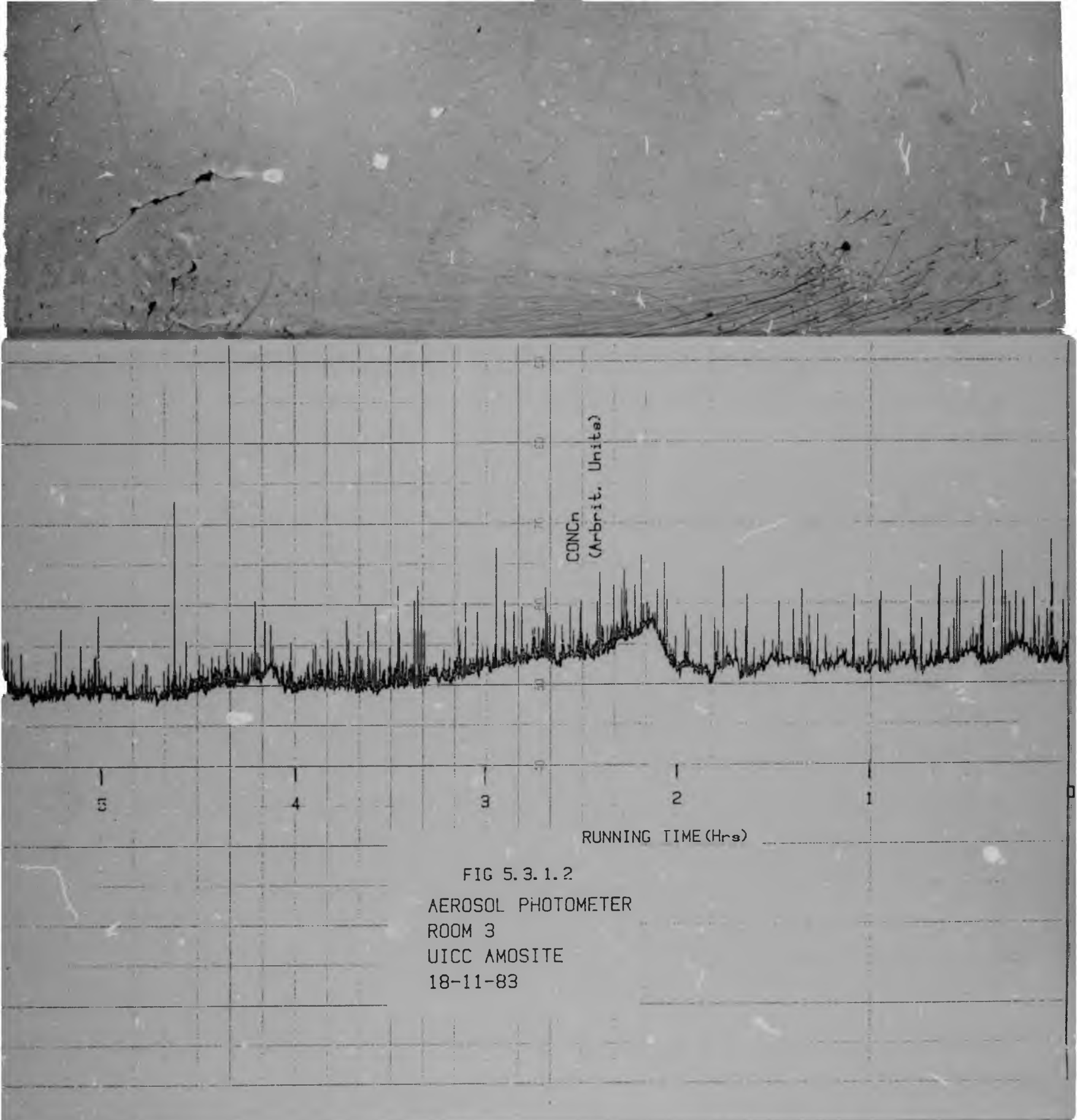


FIG 5.3.1.2

AEROSOL PHOTOMETER
ROOM 3
UICC AMOSITE
18-11-83

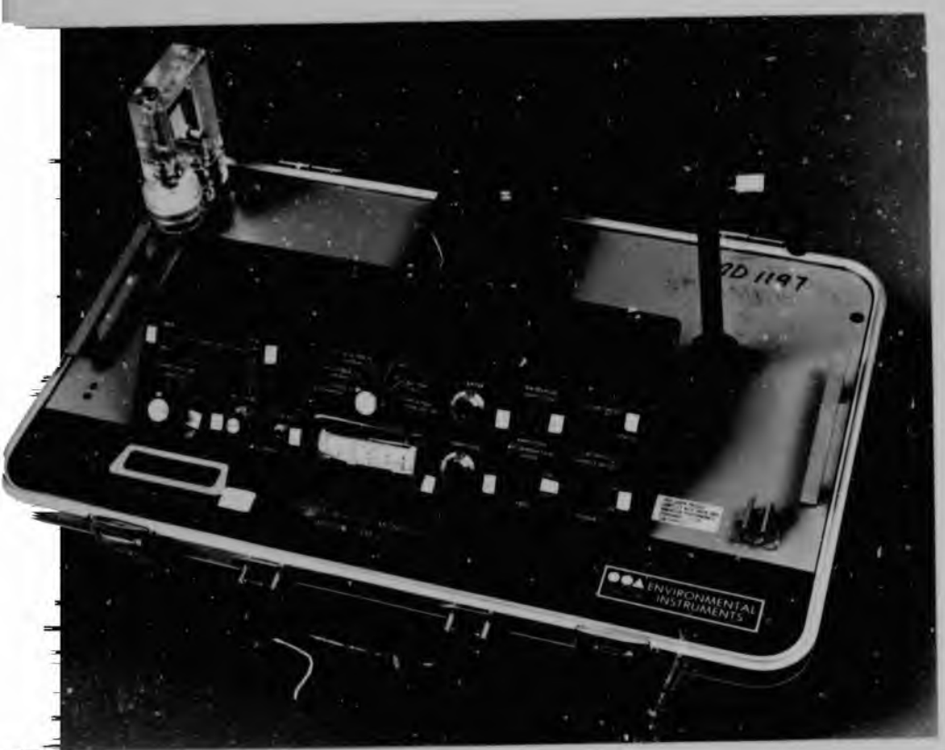


FIG 5.3.2 FIBROUS AEROSOL MONITOR

FIG 5.1.2 PERSONAL DUST SAMPLER

CHAPTER 6

EXTRACTION OF FIBRES FROM LUNGS

Even with fibre loads of many millions of fibres per gram of lung tissue one is unlikely to see a significant number of fibres in a typical histological section, firstly because the volume of the section visible under the microscope is extremely small, of the order 4×10^{-7} cubic centimetres, and secondly because the chance of a fibre lying parallel to and between the upper and lower surfaces of a 5μm section are extremely remote. The only practical method of estimating fibre load is to destroy the organic tissue and count the fibres suspended in an aliquot of the solution used.

All methods of extraction thus involve the destruction of the lung tissue and the recovery of the inorganic residue which contains the fibres. Several methods have been described including digestion in potassium or sodium hydroxide[27], bleach[28], hydrogen peroxide etc.[13,29]. Other methods which have been used with varying success are ashing in a muffle furnace[30] low temperature ashing[31] and oxidation in ozone[32].

After studying the different methods and matching their advantages and disadvantages with the equipment available at the Centre a method based on bleach digestion and incorporating some of the features of the method described by Ashcroft & Heppleston was adopted for this investigation. In previous work which involved extracting asbestos fibres from human

lungs, the laboratory had used the Ashcroft & Heppleston method as published but the bleach method was found to be much quicker and cleaner.

A diagram illustrating the whole process of extraction is presented in Fig. 6.1. For convenience this is described under the following headings.

6.1 Cutting samples from lungs

6.2 Weighing and estimating dry : wet ratio.

6.3 Digestion

6.4 Filtration

- 6.4.1 For light microscopy
- 6.4.2 For electron microscopy

6.5 Preparation for microscopy

- 6.5.1 Light microscopy
- 6.5.2 Electron microscopy

6.6 Low temperature ashing

6.7 Summary

6.1. Cutting samples from lungs

The lungs of animals which died during exposure or had been killed at some specific time after exposure were removed and stored in formalin. From these lungs samples had to be cut to allow comparisons to be made with other lungs. Statistical principles dictate that the larger the sample the more accurately it will represent the bulk from which it is drawn. However there is a limit to the size of sample which can be handled conveniently thus a compromise has to be made. This was done after an initial investigation to determine the variation in fibre concentration likely to be found in the periphery of the lung. It should be borne in mind that figures allowing comparisons between lungs were required not absolute values of the lung load. It was felt that these could be obtained by cutting portions from similar positions in all lungs.

In the lung selected for the initial investigation 9 portions were cut and their fibre loads determined. These were shown to be normally distributed (see Table 11.2 p.109) with the higher 95% confidence limit being twice the lower. This was a small range compared to the order of magnitude differences which were ultimately found in lungs which had been exposed to different fibres for different lengths of time.

Based on this finding the protocol developed for selecting samples was to cut 3 portions at random from the periphery of the lung which were then pooled to make up a final sample of 1-2g.

6.2 Weighing and estimating dry : wet ratio

Because fibre burdens in lungs are usually quoted in millions of fibres per gram of dry tissue and the preferred method of extracting the fibres is carried out on wet tissue it was necessary to determine the dry : wet weight ratio of each sample.

This was done by cutting the samples obtained in 6.1 in half and weighing each half in the wet condition. Surplus formalin was blotted off the halves before weighing. One half was then dried in a laboratory oven for 1 hour at 110°C. This had been shown adequate to drive off all (99%) the water from the specimens. (See Table 6.2) The dried half was then reweighed and the dry . wet ratio was calculated.

Table 6.2

Time in oven mins	Wt Wet Tissue (g)	Wt Dry Tissue (g)	Wt Water (g)	% Water
0	1,3648	0,1565	1,2083	100,0
15	1,1534	0,1565	0,9969	82,5
30	0,3859	0,1565	0,2294	19,0
60	0,1641	0,1565	0,0076	0,6
120	0,1565	0,1565	0,0000	0,0
240	0,1584	0,1565	0,0019	0,16

The other half of the sample was retained for fibre extraction. It was weighed and its dry weight estimated by multiplying by the dry : wet ratio determined above. Ashcroft and Hepplestone (1973)[27] have shown that the error involved in using this method was usually less than 6%.

6.3 Digestion

The first part of the fibre extraction process is the destruction of the tissue in which the fibres are embedded. This is done by digesting the tissue in hot bleach solution. The wet half of the sample obtained as described in para 6.2 above is placed in a 50ml plastic centrifuge tube and covered with about 20ml of commercial bleach (JIK) containing 3,5% $NaClO_4$; the precise volume is not important.

The tube is then placed in a hot water bath (70°). After an hour the tissue is completely destroyed and the inorganic residue, including the fibres, left as a suspension in the liquid.

Pairs of tubes are then carefully balanced and placed in a centrifuge. Centrifugation is conducted at 2500 rpm for 5 minutes. The centrifugal force at the bottom of the tube may be calculated as 1000g.

After centrifugation the supernatant liquid is decanted and replaced by exactly 50ml of distilled water. The inorganic residue is then resuspended by vigorous shaking. A typical residue from the lung of an asbestos miner is shown in Fig. 6.3.

6.4 Filtration

To obtain a sample of the fibres for examination it is necessary to remove them from the suspension by filtration. The filtration technique used depends on whether they are to be examined by light microscopy (Chapter 7) or electron microscopy (Chapter 8).

6.4.1 Light microscopy

To prepare a specimen for light microscopy 1ml of the suspension prepared in 6.3 is withdrawn using a glass hypodermic syringe and added to 10ml distilled water. The syringe is flushed a second time with distilled water to wash out any fibres which might have lodged in the needle.

The diluted suspension is then filtered through a 37mm, 0,8µm pore size membrane filter (MF) in a laboratory filter apparatus. After all the suspension has been filtered the filter and residue are dried in an oven at 110°C.

6.4.2. Electron microscopy

For electron microscopy another 1ml sample of the suspension is withdrawn from the suspension and added to 10ml distilled water.

This diluted suspension is filtered through a 37mm diameter 0,2µm pore size Nuclepore filter in a laboratory vacuum filter apparatus. After completion of the filtration the filter and residue are dried in an oven at 110°C.

When samples for special purposes such as fibre photography or determination of size distributions are

required a larger sample of the basic suspension, usually 10ml is taken and filtered. This naturally gives a higher density of fibres on the filter.

6.5 Preparation for microscopy

6.5.1 Light microscopy

For light microscopy two methods of preparation are necessary depending on whether the fibres are asbestos or glass fibre.

6.5.1.1 Asbestos fibres

Filters supporting asbestos fibres were immediately suitable for clearing and counting as described in Chapter 7.

6.5.1.2. Glass fibres

To prepare a filter carrying glass fibre for examination the following procedure is necessary.

A segment of about 30° is cut from the filter using a scalpel with a rolling motion to avoid disturbing the marginal fibres as far as possible and this is placed on a clean 75mm x 25mm glass microscope slide. It is then exposed to a stream of hot acetone vapour from a generator designed for this purpose (AIA) which softens it and makes it stick firmly to the glass slide. Once the residual acetone has evaporated the slide is placed in a low temperature asher (LTA) which is described in

Section 6.6 below. On completion of the low temperature ashing the slide is removed from the asher. A cover glass is then placed over the residue of fibres and sealed in place with a ring of beeswax round the edges[33]. It is now ready for counting under a light microscope.

6.5.2 Samples for electron microscopy

For electron microscopy both types of fibre are treated in the same way. The filters are placed in a Polaron Model 5E/100 sputter coater (see Chapter VII) and lightly coated with gold.

A portion, roughly 7mm square, is then cut out of the centre of the gold coated sample and stuck on to an aluminium specimen stub with a conducting adhesive.

A brief explanation of the necessity for this procedure is given in Chapter 8 para. 8.2.2.

6.6 Low Temperature Asher

When examining inorganic material recovered from biological specimens it is often necessary to destroy residual organic material which may be attached to it or on which it is supported - for example when it is lying on a filter. This cannot always be done by ashing in a conventional muffle furnace. The high temperatures involved may alter the inorganic particles causing sintering or changes in crystal structure or more importantly it

may cause the supporting filter to flare up or explode. This is particularly liable to happen with MF filters which consist of a thin sheet of cellulose acetate/cellulose nitrate mixture. To overcome this difficulty it is possible to destroy the organic material slowly and quietly in a low temperature asher (LTA)[34] (Fig. 6.6).

The principle of the LTA is the production of a reactive atomic oxygen plasma which will oxidize or burn away organic material at a temperature usually below 150°C. The plasma, which consists of excited O atoms and electronically activated O_2 is produced when molecular O_2 is passed through a high frequency (HF) electromagnetic field.

One such apparatus consists of a 50mm diameter Pyrex glass tube surrounded by a few turns of heavy gauge copper tubing. A radio frequency (RF) generator feeds an HF current to the coil and this creates the plasma in the tube. The pressure in the tube is maintained at about 1-2mm Hg and an air pump ensures a constant flow of air or oxygen to sweep away gaseous oxidation products. A pink or blue glow in the tube indicates the presence of the plasma. The frequency of the RF current is 13.56 MHz. The normal reaction time for a small sample such as a filter paper is 1 or 2 hours but for larger samples 2 or 3 days may be required. As the instrument gets older so the reaction time becomes longer.

6.7 SUMMARY

The number of fibres in a portion of tissue is best estimated by destroying the tissue and collecting the residual fibres. This process must be carried out with care and understanding to avoid:-

1. mechanical breakage of the fibres which will result in too high an estimate of the number of fibres in the tissue and
2. solution of the fibres which will result in too low an estimate.

The digesting reagent must therefore be appropriate to the type of fibre being extracted.

As far as possible, several samples should be taken from each specimen as the fibres are not distributed evenly throughout the specimen.

Where the lung contains a large amount of carbonaceous material in addition to the fibres, this may be destroyed by means of a low temperature plasma asher. The use of a muffle furnace is not recommended as this may damage the fibres.

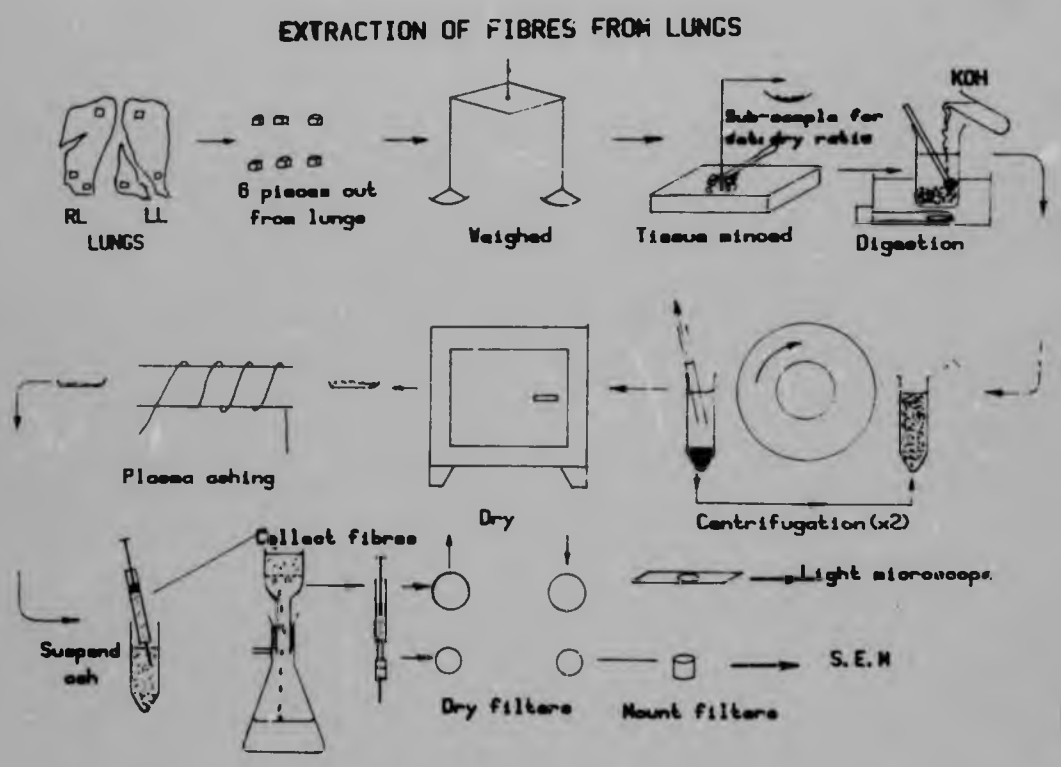


FIG 6.1 Extraction of fibres from lungs.

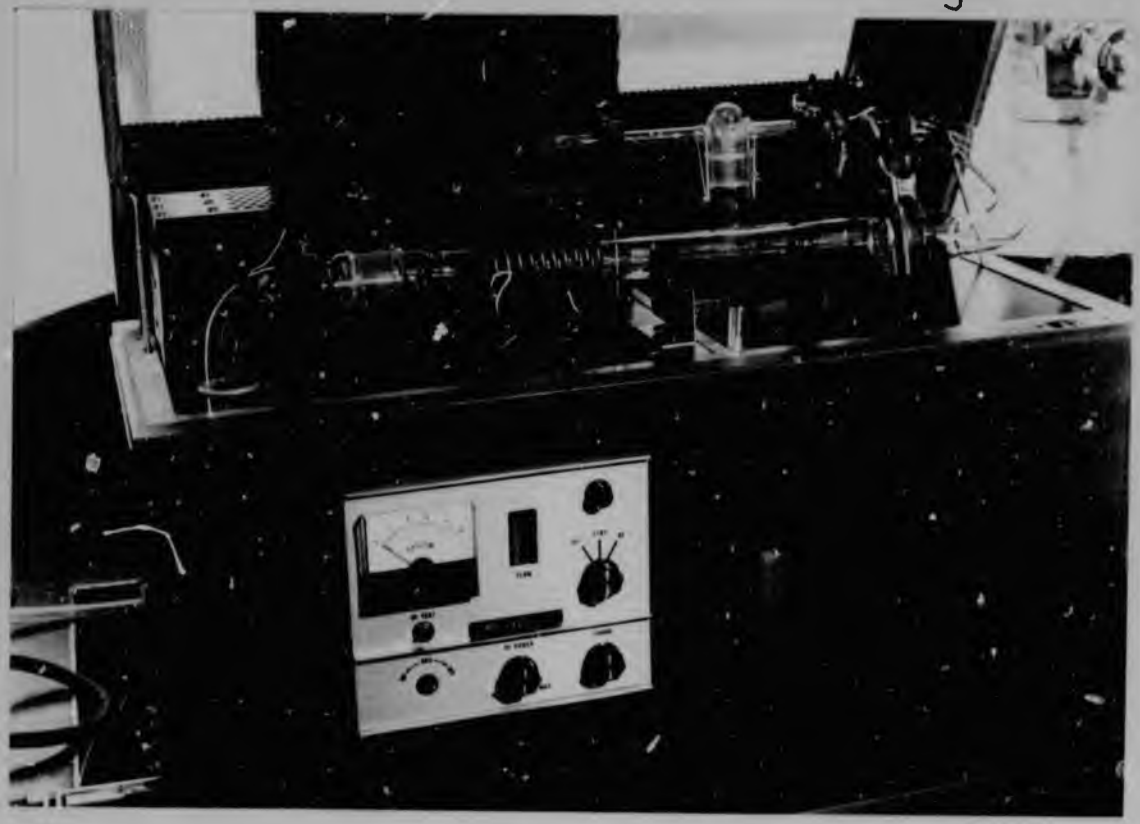


FIG 6.6 Low Temperature Asher.

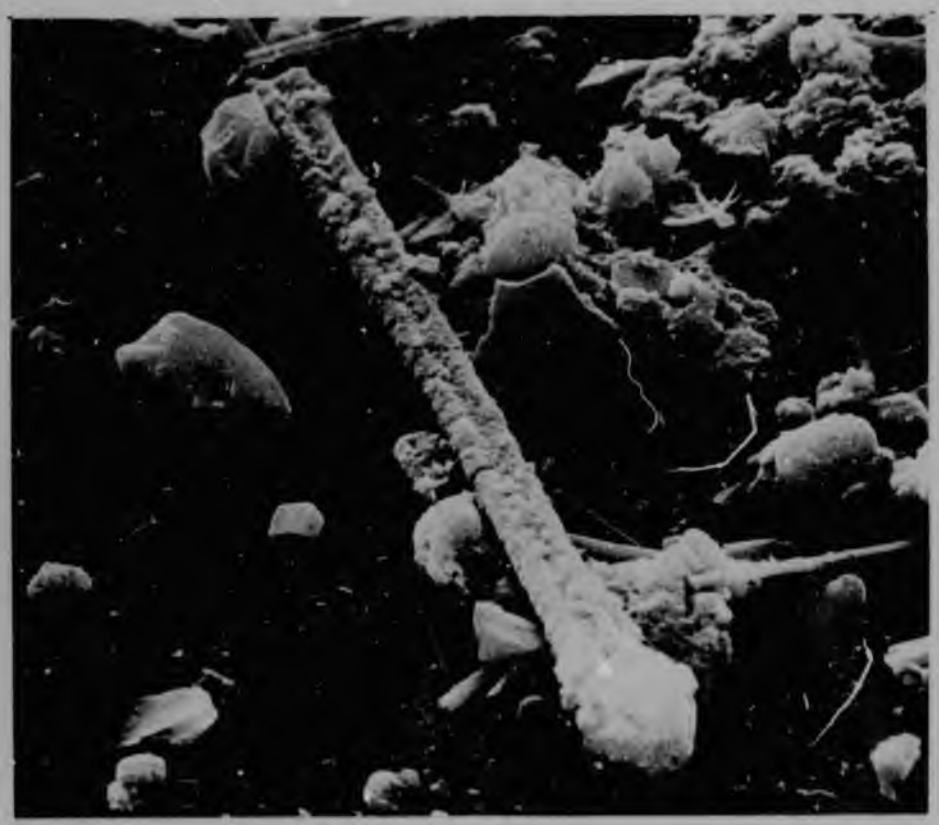


FIG 6.3 Residue from lung of asbestos miner.
(Photograph taken on Cambridge Stereoscan
Electron Microscopy Dept.
University of Witswatersrand.
(x4000)

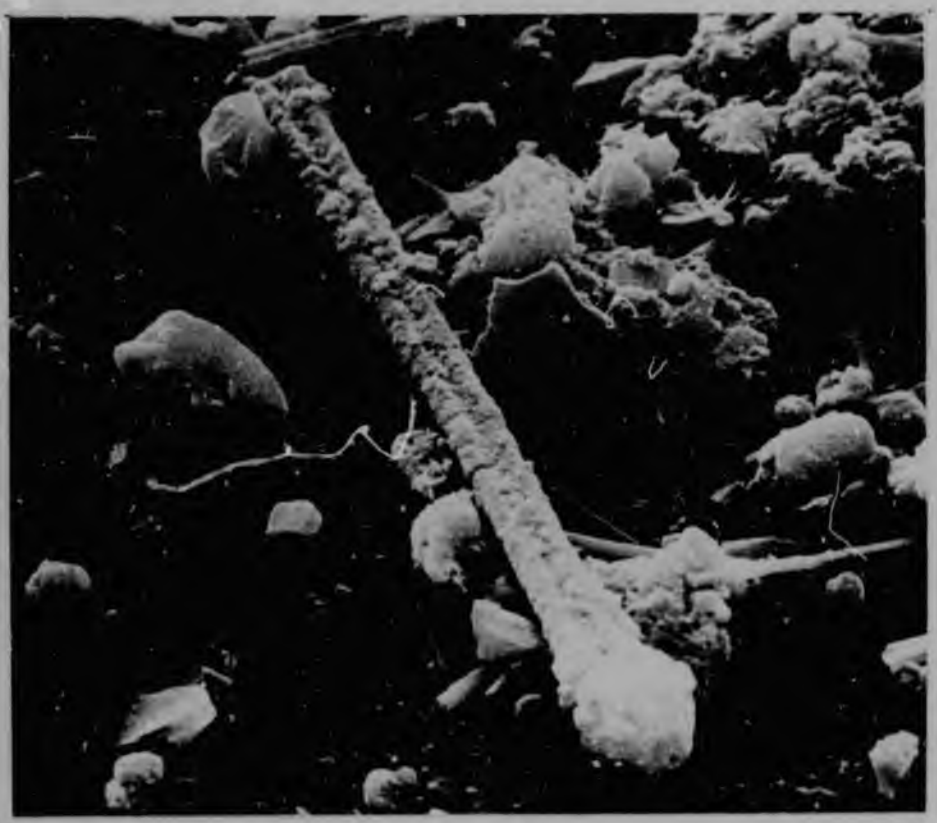


FIG 6.3 Residue from lung of asbestos miner.
(Photograph taken on Cambridge Stereoscan
Electron Microscopy Dept.
University of Witswatersrand.
(x4000)

CHAPTER 7

LIGHT MICROSCOPY

This chapter is concerned with light microscopy and counting of fibres collected from the air or recovered from the lungs of experimental animals. The subject will be dealt with under the following headings.

7.1 Filter clearing

To determine the fibre concentration in the air the fibres collected by the sampler (see Chapter 4) must be counted. Before this can be done the filter must be cleared. There are several methods for doing this[35,36,37]: the one used at NCOH being that recommended by the Asbestos International Association (AIA). The method is applicable to any type of asbestos but not to glass fibre. For a full description see AIA publication RTMI (Recommended Technical Method 1)[35].

To clear a filter, which may be either a complete 25 mm diameter filter or a segment of a larger filter, it is placed dust side up on a clean 25x75mm glass microscope slide. This is briefly exposed to acetone vapour which will soften and clear it. A cover slip with a drop of glycerol triacetate (triacetin) on its underside is then placed carefully on the preparation to make it transparent and permanent. It is generally advisable to leave the slide to stabilize for half hour before counting.

For various reasons which are discussed under Section 7.2 this technique is not wholly satisfactory for glass fibre. To overcome this difficulty a method was developed in the Occupational Hygiene Laboratory at NCOH[37] in which the acetone softened filter paper was completely destroyed in a low temperature asher leaving only the glass fibres on the slide. These fibres were then covered with a coverslip secured to the slide with a ring of beeswax.

7.2 Phase contrast microscopy

A problem with the filter clearing technique described above is that there is very little contrast between the fine fibres and the cleared filter. To enhance the visibility of the fibres a technique known as phase contrast microscopy is used. This technique converts changes in refractive index in the specimen, which are normally invisible to the eye, into changes in intensity to which the eye is sensitive. The principle of this method is best understood by consideration of Abbe's classic theory of microscopical vision[38] which is the foundation of modern light

microscope optical design. The theory explains that when a ray of light passes through an object with fine structure, as it will do when directed through a specimen on a microscope slide, it will be split into two sets of waves; direct waves from the light source and diffracted waves due to the specimen. The two sets of waves are approximately 90° out of phase with the direct waves leading. The diffracted waves are further retarded when passing through transparent objects the additional retardation being proportional to the thickness and the refractive index of the object[39].

In a normal microscope the 2 sets of waves are superimposed and no effect is noticed because the eye is not sensitive to differences in phase. In the phase contrast microscope the two sets are separated and the direct waves are advanced another 90° so that they are 180° out of phase with the diffracted ray. The rays are then recombined and, because they are 180° out of phase, the resultant ray will be reduced in amplitude and hence in brightness since brightness is proportional to the square of the amplitude. In this way the microscope has converted differences in refractive index in the specimen and between the specimen and the field into changes in intensity. See Fig. 7.2.

7.3 Fibre Counting

7.3.1 The microscope

Any good quality research microscope fitted with phase contrast accessories is suitable for fibre counting. If possible its performance should be checked against the HSE/NPL test slide (see par. 7.3.2) before purchase.

The minimum optical requirements are a x10 Phi objective, a x40 Ph2 objective and x12 compensating eye piece. The prefixes Ph 1&2 identify the objectives as being fitted with phase plates making them suitable for phase contrast microscopy. Some binocular microscopes incorporate a x1,5 magnification in the optical system which deflects the light coming vertically up the tube into the eye piece tubes. In this case x8 compensating eyepieces should be used.

7.3.2 Test Slide

Before starting to count fibres the microscope must be checked to ensure that the optical system is properly aligned and is giving the required contrast. The main alignment is to position the white annular diaphragm in the condenser under the grey annular phase plate in the objective. This is done by observing the two rings with a miniature telescope inserted into the eyepiece tube and manipulating two controls on the stage.

When the microscope is correctly adjusted a test slide[35] designed by the Health and Safety Laboratory in U.K. and replicated from a master slide produced by the National Physical laboratory Teddington (HSE/NPL test slide. See Appendix B) is put on the stage and examined with the objective to be used. The test consist of resolving as many as possible of 7 sets of parallel lines of decreasing fineness and contrast drawn on a 75x25mm

microscope slide. The objective should resolve set number 5 at least if it is to be suitable for fibre counting. If set no 5 is not resolved the optical system should be cleaned, realigned and tested again. Failure to resolve the test slide after cleaning and realigning probably means that the microscope is not suitable for fibre counting. Details of the test slide are indicated in Appendix B.

7.3.3 Eyepiece graticule

Fibre counting is done within the bounds of a graticule placed in the eyepiece. The graticule is engraved on a glass circle which is placed on the field stop within the eyepiece. It may be necessary to unscrew the top lens of the eyepiece to bring the graticule into focus.

Various types of graticule have been described in the past but the one most commonly used today is the circular graticule of Walton and Beckett (Fig. 7.3.3)[40]. This graticule subtends a circle of 100μm diameter on the field being examined. Round the circle are simulations of fibres of different lengths and diameters which assist the microscopist to decide whether a fibre should be counted or not. (See par. 7.3.4 below)

7.3.4 Counting Rules

To begin with not all the fibres collected on the filter are considered biologically harmful. By convention[35] only those fibres between 5 and 100μm long, less than 3μm diameter and having an aspect ratio greater than 3:1 are counted. This convention is set out diagrammatically in Fig. 7.3.4. Along the bottom, or x-axis are the lengths of fibres one might expect to find airborne, while up the y-axis is a typical range of diameters. Fibres of any combination of length and diameter might be found in the air but by convention only those in the shaded area are counted. This convention is a compromise between that which is desirable and that which is practicable.

A second restriction on counting is the position of the fibre relative to the image of the graticule. All fibres in the countable range and completely within the graticule are counted but how to count those partly in and partly out of the graticule has been the subject of much debate. The currently preferred method is to count them as half a fibre. Though this is a somewhat laborious procedure it is considered to be the most statistically unassailable[41].

7.3.5 Limit of resolution

The diameters of airborne fibres decrease continuously from what is clearly visible under the microscope to less than the wavelength of light. Fibres therefore gradually

appear fainter as they become finer until they are completely invisible. A considerable proportion of the fibres lie within this invisible range. To establish the boundary between the visible or microscopic fibres and the invisible or sub microscopic the following simple study was carried out.

A suspension of crocidolite, a fine asbestos, was smeared on a glass slide. Suitable fields were selected under the light microscope and marked with black ink circles.

The slide was then prepared for scanning electron microscopy (SEM) by lightly coating it with gold to make it conducting. The thickness of the gold coat as judged by the finest fibres visible was less than 0,0025µm. SEM micrographs of the selected areas were taken at X3000. From the negatives X5000 prints were made. The slide was then removed from the electron microscope and transferred to the stage of the light microscope.

The selected areas were brought into the field of view of the microscope and fine fibres in the SEM photographs, selected at random, were sought in the field of view of the light microscope. The photograph was marked to indicate which fibres were visible and which fibres were not. One such photograph is included as Fig. 7.3.5.1.

Finally the diameters of the fibres on the SEM photographs were measured under a low power (X10) stereo dissecting microscope fitted with a millimetre scale subdivided into 1/10mm. With this arrangement it was possible to measure fibre diameters to within 0,02µm. The real limit of accuracy was the graininess of the print nevertheless there are many fibres in Fig. 7.3.5.1 which are not visible to the naked eye. They can only be seen with X10 magnification. It was not necessary to measure their diameter as they were clearly far below the resolution of the light microscope. The fibres were then divided into size ranges 0,02µm wide and the number of fibres marked visible or not visible under the light microscope were counted in each range.

The ratio of visible to total for the different size ranges has been plotted as a histogram in Fig. 7.3.5.2. Since only fibres greater than 0,32µm could be detected with a 100% certainty this was assumed to be the limit of resolution of the light microscope.

Fibres less than 0,32µm are counted using the scanning electron microscope as discussed in the next chapter. A similar investigation has been reported by Middleton[41].

7.3.6 Counting procedure

There are many different ways of counting fibres on a filter and it is important to decide early on in an

experiment what procedure should be used and which then should not be varied.

The procedure adopted in this investigation was to count the fibres in 30 eyepiece graticule areas selected at random from all over the filter.

The fibres were divided into 2 classes, coated and bare, and separate counts were made of each class. Coated fibres are those which have acquired a layer of ferruginous protein round them during their residence in the lung. Fig. 7.3.6. shows a typical residue from a baboon's lung and includes both bare and coated fibres.

The counts were recorded on special counting sheets designed to reduce the labour and possibility of error when entering the data into the computer. The computer did the tedious calculations necessary to convert the microscope counts into fibres per gram of dry tissue in the case of lung samples and fibres per cubic centimetre of air in the case of air samples. An example of such a sheet is shown in included as Appendix C(1). They effectively reduced the number of computer entries to be made by grouping similar fibre counts together. Appendix C(2) is a similar sheet designed for use when counting under the electron microscope.

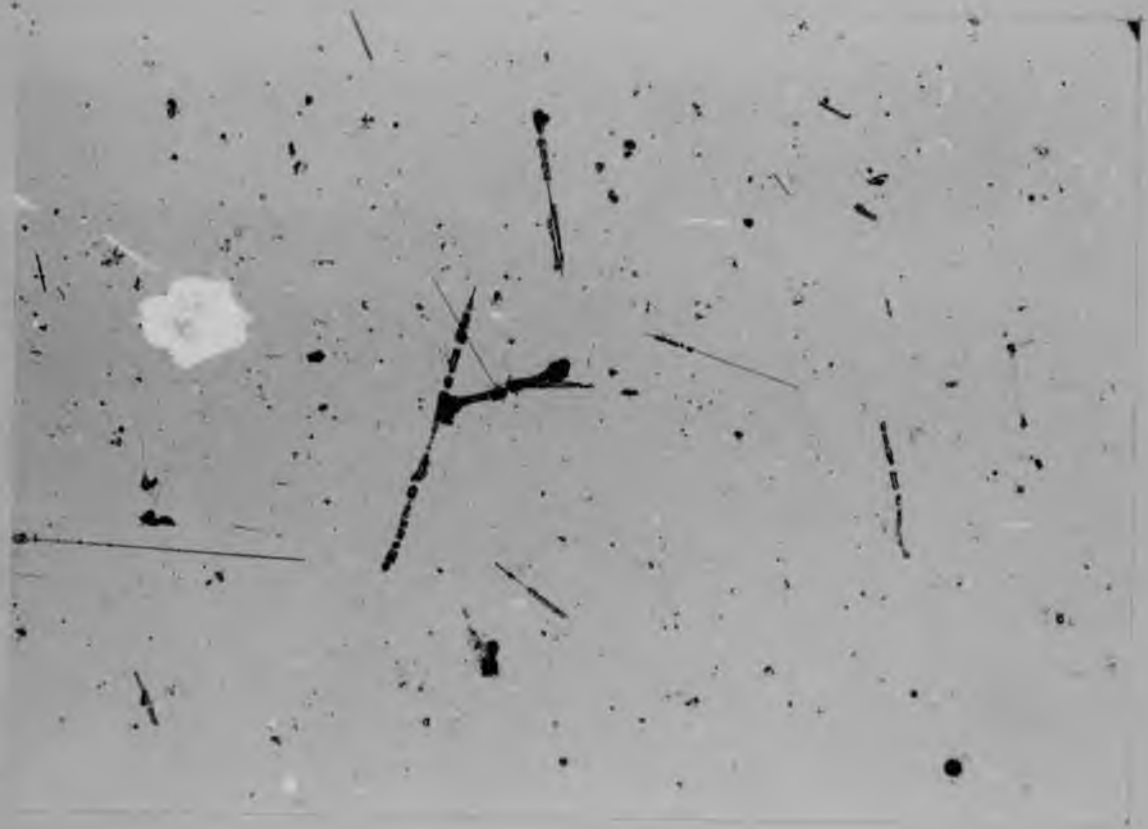


FIG 7.3.6 Light micrograph of baboon lung residues. (x400) Note bare and coated fibres

FIG 7.3.6 Light micrograph of baboon lung residues. (x400) Note bare and coated fibres

As explained in par. 7.3.5 many of the fibres collected on the filter are too fine to be observed under the light microscope. The procedure for counting these is described in the next chapter, Electron Microscopy.

7.4. SUMMARY

Light microscopy is an essential part of the procedure to estimate the number of fibres in a known weight of lu tissue. To prepare a sample for microscopy the fibres must first be extracted from the tissue, then suspended in an appropriate volume of liquid and then spread out evenly on a transparent base for counting.

The last part of this procedure involves filtering the suspension through a special filter to collect the fibres. The filter is then dried and supported on a glass microscope slide, rendered transparent by special clearing liquids and covered with a cover slip. However because of the refractive index of the clearing liquids used there is poor contrast between the fibres and the background and they cannot be seen easily. To make them clearly visible a phase contrast microscope must be used. This converts small differences in refractive index which are not seen by the eye into differences of tone which are.

A graticule is fitted in the eye piece of the microscope to define known areas of the filter and by counting the fibres enclosed by a known number of these areas (usually 50) the number of fibres on the filter may be estimated. From this figure the total number of fibres in the original block of tissue may be calculated. It is usually quoted in millions of fibres/gram of dry tissue (Mf/g).

A light microscope is unable to resolv all the fibres on the filter as many have diameters less than the wavelength of light.

To identify these fibres it is necessary to resort to electron microscopy.

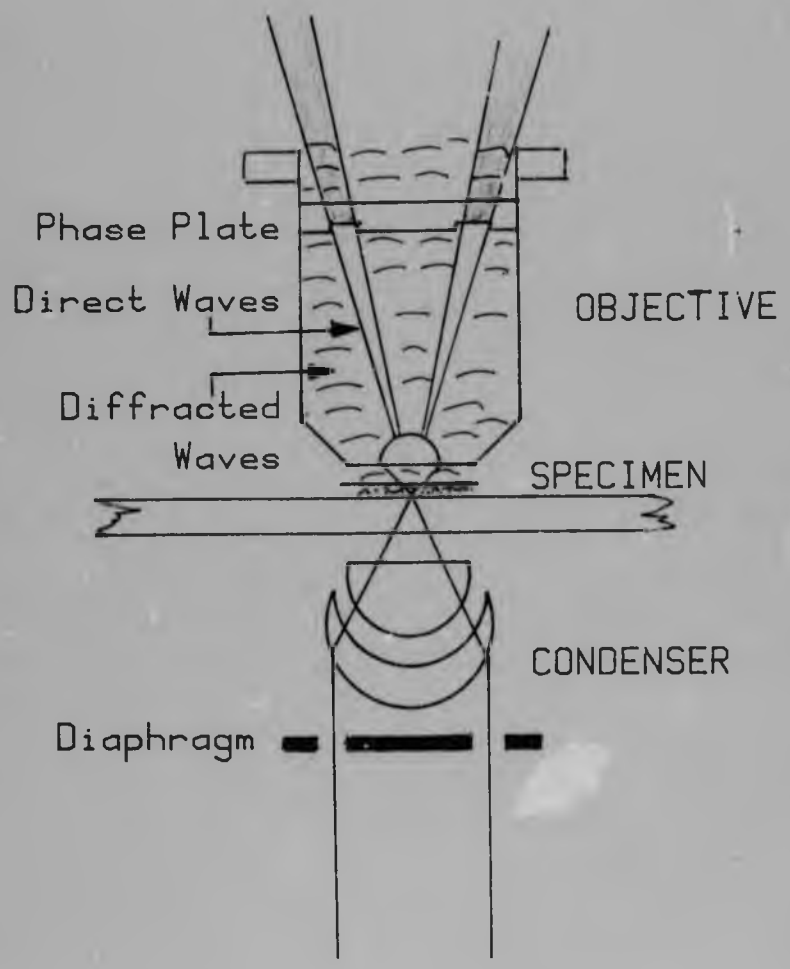


FIG 7.2 Principle of Phase Contrast microscopy.

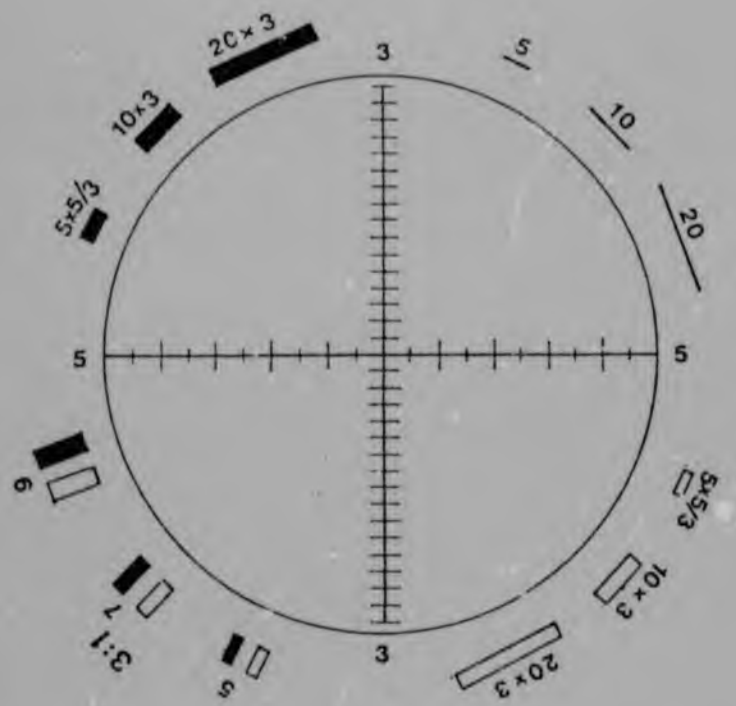


FIG 7.3.3 WALTON & BECKETT fibre counting graticule.

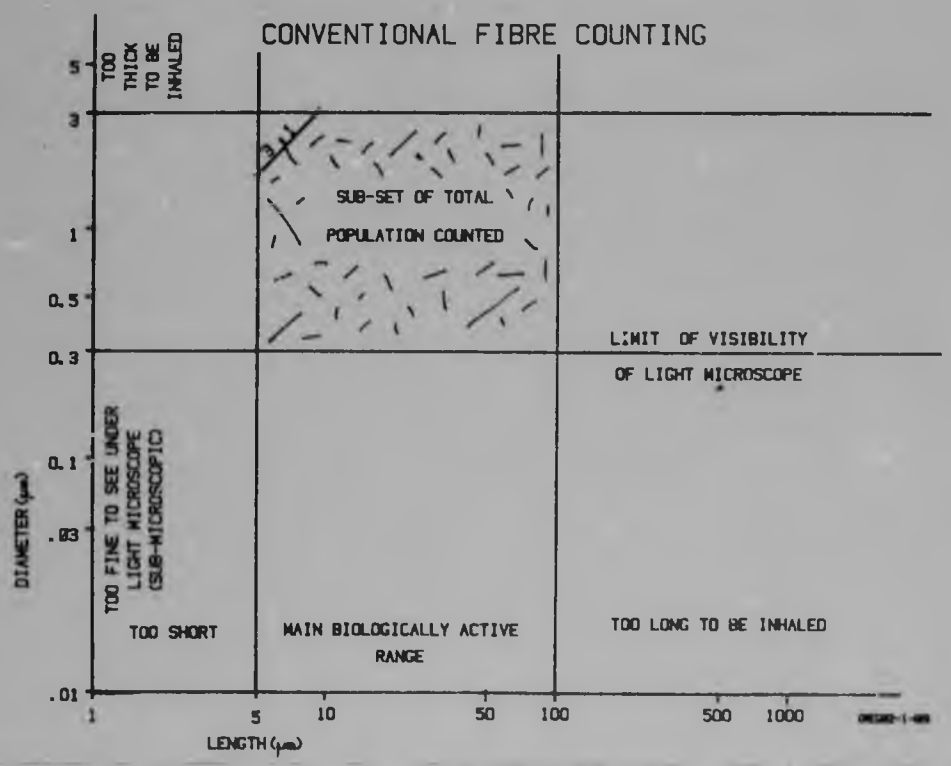


FIG 7.3.4 Fibre counting conventions.

FIG 7.3.5.1 SEM photograph used to determine limit of resolution of light microscope. Fibres circled were invisible under LM.

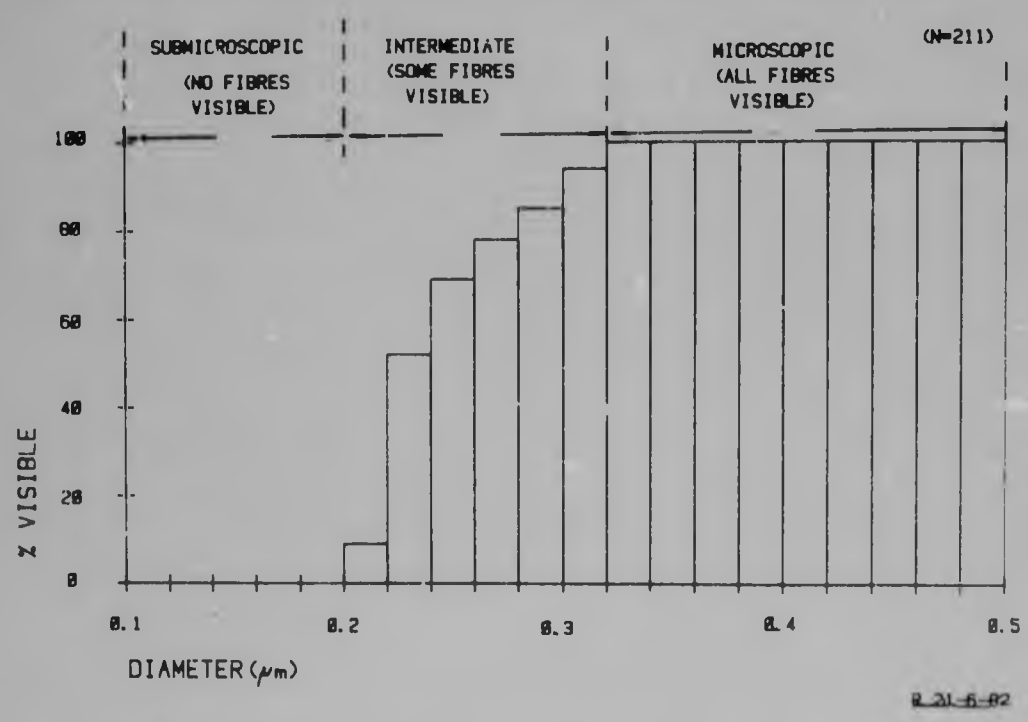


FIG 7.3.5.2 Boundary between visible and invisible fibres under light microscope (LM).

CHAPTER 8

ELECTRON MICROSCOPY

Electron microscopy is required to see particles or fibres too fine to be resolved by the normal light microscope. Many fibres, in some cases the majority of the fibres, fall into this category so electron microscopy is essential in any study of respirable fibres. In an electron microscope the specimen is irradiated by a beam of electrons instead of a beam of light. This increases the resolution of the system as the effective wavelength of the electrons is very much shorter that that of light. See Fig. 8.1.

Broadly speaking electron microscopes (EM) may be divided into 2 categories:

8.1 Transmission E.M.'s suitable for observing objects thin enough to allow a beam of energy to pass through them and thin sections showing the internal structures of thicker specimens.

8.2 Scanning E.M.'s to observe the outside structure of specimens.

In this study only the latter type of E.M. was used so only those techniques in which it was involved will be discussed. However it is convenient to discuss the importance of wavelength in image formation under transmision electron microscopy.

8.1 Transmission electron microscopy

The importance of wavelength in image formation

The higher resolution of the electron microscope arises from the very short wave length of the fast moving electrons consituting the beam. The relationship between the high speed electrons and their wavelength λe, (de Broglie wavelength) is expressed by the following equation:

$$\lambda e = \frac{h}{m_o v}$$

h = Planck's constant

6.6×10^{-34} J-secs

m_o = electron mass at rest

$9,11 \times 10^{-31}$ kg

v = velocity of electron

1×10^{8}

Substituting typical values in this expression. the wavelength of the electrons is found to be of the order 0,01nm. This should be compared with 700-500nm for visible light.

The importance of wavelength in forming images in transmission electron microscopy (TEM) is shown in Fig. 8.1.1.

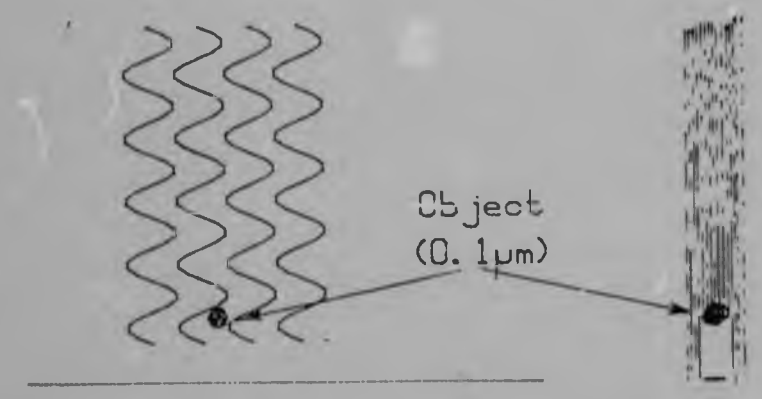


FIG. 8.1.1.

A similar situation prevails in scanning electron microscopy (S.E.M.) where the resolution depends on the beam diameter or 'Spot' size. This generally has to be of the order 100—50nm far below the wavelength of light. The principle limiting the size of the spot to which wave radiation may be focussed is shown in Fig. 8.1.2.

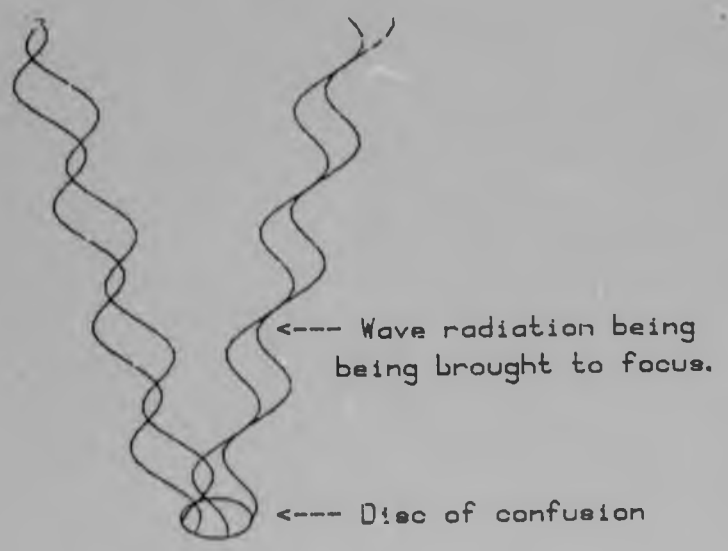


FIG. 8.1.2

The ultimate spot is called a 'disc of confusion' or Airy disc whose diameter may be calculated from the relationship

$$2h = \frac{1,22\,\lambda}{N.A.}$$

h = radius of disc

λ = wavelength of radiation

N.A. = Numerical Aperture

From this equation it can be seen that resolution does not only depend on the wavelength of the radiation it also depends on the numerical aperture (NA) of the optical or electronic system.

The numerical aperture whether optical or electronic is a measure of the energy gathering power of the system or its ability to collect rays at a wide angle (u) from the central ray. This in turn is an important factor in determining resolution (see equation below). This concept can be best understood from Fig. 8.1.3.

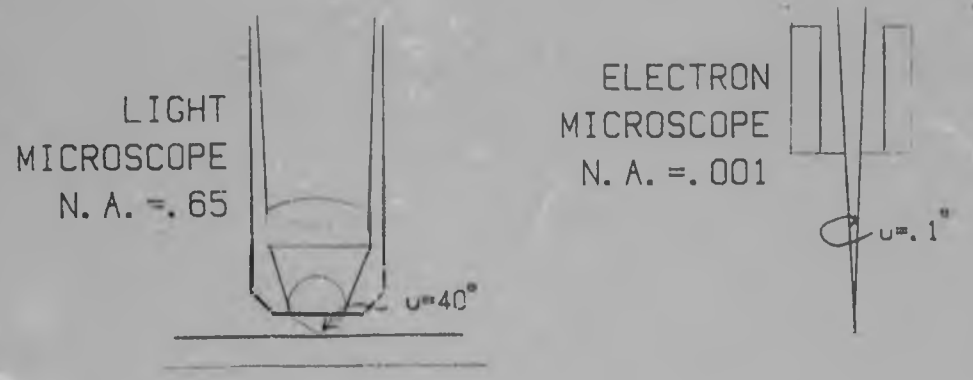


FIG. 8.1.3. NUMERICAL APERTURE OF LIGHT AND ELECTRON MICROSCOPES

Mathematically N.A. may be defined by the following relationship

N.A. = n Sin u n = refractive index of surrounding medium (= 1 for vacuum, 1.003 for air)

u = 1/2 apical angle of light cone

and resolution h as

$$h = \frac{0,61\,\lambda}{N.A.}$$

This is the closest two Airy discs radius h can be to be seen as two separate points. If they are closer they merge and are seen as one.

ubstituting typical values in this formula we find for

1. Light microscope

$$h = \frac{0,61 \times 550 \text{ nm}}{0,65}$$

550nm = mean wavelength of light

0,65 = N.A. of x 40 obj.

= 516nm

and for

2. Electron microscope

$$h = \frac{0,61 \times 0,01 \text{ nm}}{0,001}$$

0,01nm - equivalent wavelength of electron

0,001 - Typical NA of EM

= 6 nm

i.e. the resolution of the E.M. is approximately 80 tin s that of the light microscope.

8.1 Transmission electron microscopy

Since transmission electron microscopy was not used in this study, it is not considered relevant to discuss its principles and techniques here.

8.2 Scanning Electron Microscopy

Scanning electron microscopy may be considered under the following headings.

8.2.1 Use of the microscope

8.2.2 Specimen preparation

8.2.3 Fibre counting

8.2.3.1 Counting on screen

8.2.3.2 Taking photographs

8.2.1 Use of the microscope

The principle of the scanning electron microscope is that a fine beam of electrons is scanned over the specimen by means of scanning coils within the final lens of the column. The scan is not continuous but is made in a series of 'steps'. At each 'step' the beam reacts momentarily with the specimen causing it to emit a wide variety of radiation. In particular secondary electrons, back scattered electrons and X-rays are emitted. Any of these emissions may be collected using an appropriate detector. For image formation secondary electrons are collected. For microprobe analysis, the beam is held stationary on one spot and X-rays are collected.

The scan generator controlling the scanning of the object is also connected to the viewing screen scanner and each position on the object is in a 1:1 relationship with a position on the screen. Also the intensity of the image on the screen is proportional to the emission from the specimen. Thus by suitable scaling up, the image on the screen can be made an enlarged replica of the object within the microscope.

To obtain a satisfactory image two opposing influences have to be reconciled. Sharp definition requires a fine incident beam of electrons but a fine beam can only carry low power which results in poor emission of secondary, or image forming electrons. The skill of the operator is manifest in the manner in which he balances these two opposing requirements. Clear and detailed instruction for operating the S.E.M. when viewing asbestos and other inorganic fibres are set out in AIA RTM2.[51]

There are two other methods of displaying the image besides the viewing screen. These are a TV screen and photographs. Each display has its own particular advantages. The viewing screen image is fine grained but dark and transient. The TV image is coarser grained and bright while the camera, either Polaroid or conventional records a permanent fine grained image. Naturally colour is not available as the image is formed by an invisible electron beam.

The microscope used in his investigation was a Jeol 25S fitted with a KEVEX 7000 microprobe.

8.2.2 Specimen Preparation

Specimens for examination in the S.E.M. are mounted on 10mmx10mm cylindrical aluminium stubs with a suitable conducting adhesive such as silver DAG.[42] This ensures that they remain at earth potential and prevents a charge building up on them due to the stream of electrons. If a charge does build up due to faulty earthing the beam will be repelled and the image will fade. To conduct the electrons away from the surface of the specimen it is necessary to coat it with a fine conducting layer.

The coating used in this work was gold. This provides a good, inert, highly conducting surface on the specimen but has the disadvantage that it interferes with any analysis by the microprobe. If microprobe analysis to identify fibres is anticipated a carbon coat has to be used.

Gold coating is carried out in a 'sputter' coater. In this apparatus the specimen forms the anode and the target or source of coating material forms the cathode of a sputter system. When a high voltage, 2-3kv, is applied between the two electrodes under vacuum conditions a stream of target atoms is emitted on to the specimen covering it with an even coat of metal.

The sputter coater used in this work was Polaron Model 5E/100.

8.2.3 Fibre Counting

Fibres may be counted either directly on the viewing screen of the S.E.M. or from photographs. The first procedure is usually adequate when only a fibre count or concentration is required but when a full size distribution is required, in addition to the concentration it is usually more satisfactory to employ the latter.

8.3.2.1 Counting fibres directly from the screen

This was done at x2000 magnification. At this magnification a fibre 1µm diameter appears 2mm wide and the limit of resolution is about 0,1µm.

Counting is done in much the same way and applying the same rules as explained under par. 7.3.4 for light microscopy. Fibre dimensions may be measured directly using a scale marked with distances representing microns at x2000 on the microscope screen. The number of fibres counted in a known number of fields of view is the information required to calculate the fibre concentration.

Expressed mathematically this appears as follows

$$C = \frac{A.N.1}{a\ n\ v}$$

Where C = concentration (f/cc)

A = effective area of filter (mm^2)

a = area observed under SEM (mm^2)

N = Number of fibres counted

n = Number of fields of view

v = Volume of suspension or air sampled (ml)

The field of view of the microscope at X2000 was 0,002mm^2.

8.2.3.2 Fibre counting from photographs

Measuring fibres directly on the viewing screen is a tiring procedure for which microscope is not ergonomically designe' Where a large number of fibres have to be counted and measured as will be necessary when determining the size distribution of the fibres it is far more practical to take a series of photographs and measure from them. The concentration of fibres on the filter should be 10-100x greater than when counting from the screen.

Counting and sizing may be done either manually or, through a digitizing platen. by a computer. When counting manually it is usually only practical to base size categories on one dimension of the fibre, either the length or the diameter. With the digitizer/computer

combination it is possible to classify by two dimensions, length and diameter, simultaneously.

Since the majority of fibre lengths or diameters are log normally distributed or approximately so, it is most convenient to classify the fibres into cells or categories whose higher bound is twice the lower bound. This is equivalent to a constant cell width of 0,3 on the log scale. Typical classification schemes would be as follows:

LENGTH

Length(µm):	2,5-5	5-10	10-20	20-40	40-80	80-160
Log Length:	0,4-0,7	0,7-1,0	1,0-1,3	1,3-1,6	1,6-1,9	1,9-2,2

DIAMETER

Diam(µm):	0,09-,18	,18-,37	,37-,75	,75-1,5	1,5-3,0
Log Diam:	-1 -0,7	-0,7 -0,4	-0,4 -0,1	-0,1 0,2	0,2 0,5

Using the normally distributed log values it is possible to carry out a number of standard statistical tests on data or sets of data.

8.3 SUMMARY

The electron microscope is necessary to see fibres whose diameter is less than the wavelength of light and there is a high proportion of these fibres in a typical dus cloud. Broadly speaking there are two categories of electron microscope (E.M.) the transmission E.M.s (T.E.M.) used to examine the internal structure of specimens through the use of ultra thin sections and scanning E.M.s (S.E.M.) used when the external morphology of the specimen is of interest. In this study an S.E.M. was used.

Although the S.E.M. provides a far higher magnification (x100 000) than the light microscope it is more difficult to use. Counts can be made directly from the screen over the areas covered by the field of view at any magnification but other measurements are best made from photographs.

The preparation of the specimens is more complicated and time consuming than specimens for the light microscope.

CHAPTER 9

COMPUTING

INTRODUCTION

A technical desk top computer system was used to collate the raw data and present the final results of this study. A high powered "number cruncher" was not required, it was more important to have a modest machine which was simple to operate and over which one had complete control. With a system such as this it was possible to try out different methods of analysis and presentation quickly.

Both commercially available and specially written programmes were used. These programmes are discussed more fully below. An important feature of the computer was that it was easy for a non-specialist to programme.

The computer system and its applications are described under three headings:

9.1 Equipment

9.2 Programmes

9.3 Summary

9.1 Equipment

9.1.1 The computer

The computer was a Hewlett Packard HP85A portable desk top computer. Its performance and capacity had been enhanced by the addition of a 16k memory module which brought the total RAM (Random Access Memory) up to 32k.

Although this was scarcely enough for some programmes it was adequate most of the time. To further expand the capabilities of the system a ROM (Read only memory) drawer was fitted to carry the ROMs necessary to control the following peripherals.

9.1.1.1 Double disc drive mass data storage

The use of a disc instead of the tape with which the computer was originally fitted greatly speeded up data storage and changing from one programme to another.

9.1.1.2 A4 Flat bed plotter

The majority of the graphs and diagrams used to illustrate this dissertation were drawn on this plotter under computer control.

9.1.1.3 Digitizer

A digitizer is the reverse of a plotter - it converts graphical information into digital information. This allowed dimensional data, for example lengths and diameters of fibres to be fed directly into the computer from micrographs or electron micrographs (Fig. 7.3.5.1). With this facility size distributions based on fibre length or diameter could be produced quickly. More complex 2 dimensional distributions based on length x diameter together could also be produced. See Appendix A. These are very laborious to create manually.

Although this was scarcely enough for some programmes it was adequate most of the time. To further expand the capabilities of the system a ROM (Read only memory) drawer was fitted to carry the ROMs necessary to control the following peripherals.

9.1.1.1 Double disc drive mass data storage

The use of a disc instead of the tape with which the computer was originally fitted greatly speeded up data storage and changing from one programme to another.

9.1.1.2 A4 Flat bed plotter

The majority of the graphs and diagrams used to illustrate this dissertation were drawn on this plotter under computer control.

9.1.1.3 Digitizer

A digitizer is the reverse of a plotter - it converts graphical information into digital information. This allowed dimensional data, for example lengths and diameters of fibres to be fed directly into the computer from micrographs or electron micrographs (Fig. 7.3.5.1). With this facility size distributions based on fibre length or diameter could be produced quickly. More complex 2 dimensional distributions based on length x diameter together could also be produced. See Appendix A. These are very laborious to create manually.

9.2 Programmes

Two types of programmes were used.

9.2.1. Standard commercially available programmes

9.2.2. Specially written "in-house" programmes for specific purposes.

9.2.1 Standard programmes

9.2.1.1 'CURVE' in the HP Standard pac was used to fit different types of regression curve to the plotter data. This was the most important and extensively used commercial programme. Examples of the use of this programme are the regression equations used in Figs. 10.1, 10.2, et seq.

9.2.1.2 'ONESAM' in the HP General Statistics manual was used to determine the means and other statistical parameters of, for example, the airborne fibre concentrations of the different types of fibre. (See Table 4.1)

9.2.1.3 'PAIRED' Also available in the HP General Statistics pac was used for analysis of various regression equations.

9.2.2 Specially written 'in house' programmes

These were programmes written to carry out specific calculations required in this investigation or for use when

the commercial programmes were not quite suitable. The four most important of these programmes are described below.

9.2.2.1 'FPCC' This was a most important programme used to calculate the fibre concentration in the air of the dust rooms or in the liquid suspensions after digestion of lung tissue. It was used in conjunction with a specially designed form, Appendix C(1), on which the results of the fibre counts made under the microscope were recorded. This form was designed to take the fullest advantage of the computer's calculating speed by reducing the number of entries which had to be made.

At the same time as the fibre count was evaluated the numerical density of fibres collected on the filter (measured in fibres per square millimeter of filter surface (f/mm^2) was also calculated. This affects the precision of the final result. If the density of fibres on the filter is too low ($<100/mm^2$) or too high ($>1000/mm^2$) the confidence limits on the counts are likely to be wider than if the count lies between these two values. This figure is not usually determined when fibre concentrations are calculated manually consequently it is not possible to gauge the likely precision of the result.

9.2.2.2 'PLTGRF2' was used to plot the data on A4 paper. It allowed considerably more flexibility in the layout and lettering of graphs than the commercial or standard programmes. Figures 10.2.1.1 and 10.1.1.2 etc were drawn using this programme.

9.2.2.3 'LABELS' This was a programme to write neat lettering of a wide range of size in any direction in any position on a graph or diagram. It overcame the problem of writing headings and subsidiary notes on diagrams which is an art in itself and which, if carried out imperfectly can mar an otherwise technically perfect drawing. At best lettering is a difficult and tedious job. All the lettering on the diagrams and graphs in this disseration was done using this programme.

9.2.2.4 'REGCONLIM' This programme was written to calculate and draw the 95% confidence limits on the slopes of the regression lines used in Figs. 10.1. and 10.2. The formulae involved, though simple in concept would be excessively time consuming to evaluate manually.

9.3 Summary

This chapter has shown how a small desk top computer system can be used to do many of the tedious and time consuming operations in evaluating the results of an investigation.

The importance of being able to write programmes to carry specific tasks was also stressed. The commercially available programmes can only approximate to one's requirements and to get exactly what is wanted, one must be able, through a programme, to give the computer precise instructions.

Finally computers with vast amounts of memory are not always needed for technical work; simplicity of programming is more important.

CHAPTER 10

RESULTS

In this Chapter the results of 4 related studies are presented, namely

10.1 Glass fibre retention and clearance

10.2 Crocidolite clearance

10.3 Amosite retention and clearance

10.4 Chrysotile retention

The results are summarized and compared in a Summary (10.5) at the end of the chapter.

10.1 Glass fibre

The data collected during the exposure to glass fibre are set out in two Tables. Table 10.1.1 which shows the data related to the build up of the glass fibre concentration in the lungs of the animals during the period of exposure and Table 10.1.2 which shows the decrease of fibre concentration during the survival period after exposure.

10.1.1 Build-up study

Table 10.1.1 was compiled from the data obtained during the examination of the tissue from 5 baboons which died during exposure (See. Fig. 3.3.1) and a control baboon which was not exposed to the fibre. Two estimations of the fibre load were made on each lung specimen except no 404 for which three were made. Only one fibre estimation was made on the control.

In the table the higher figures have been rounded off to the nearest million for the sake of clarity and in consideration of the degree of accuracy obtainable in this type of work.

Table 10.1.1

"Build-up" Study : Glass Fibre

Baboon No	Exposure (Months)	Survival (Months)	D:W Ratio	Mf/g Wet	Mf/g Dry	Mean Dry	Notes
404	14	0	0,07	2 9	32 111 206	116	R.U R.L. Random
405	19	0	0,18	119 118	661 656	659	Random Random
406	16	0	0,28	166 198	593 707	650	Random Random
407	6	0	0,08 0,01	30,8 24,4	385 244	315	R.U. L.U.
408	12	0	0,08	20,9	261 555	408	R.U. L.U.
488	Control		0,15	0,01	0,07	0,07	Random

10.1.1.1 Explanation of table

Column one of the table shows the number of the baboon. Baboons are numbered serially as they are brought into the animal house of the Centre and a

complete record of their history while at the Centre is maintained. Column 2 records the period of exposure to the dust in months. The D:W ratio is the ratio of the dry weight of a specimen to its original wet weight (See Chapter 6 para 6.2). Columns 5 and 6 contain the values of the fibre concentration found in the lung material expressed as millions of fibres/gram of wet or dry tissue (Mf/g). The actual determination is made on wet tissue and this is converted to a value based on dry tissue by dividing by the Dry:Wet ratio. Fibre loads are generally expressed in Mf/g dry tissue to eliminate one variable, i.e. moisture content from the results and also to allow better comparisons to be made between laboratories. Column 7 shows the mean value of the dry fibre concentration where several estimations have been made on the same specimen. This is the value plotted on the graph.

The Code in the last column refers to the lobe of the lung from which sample was taken.

RU Right upper

RL Right lower

LU Left upper

LL Left lower

Random - used where the lobe was not specified.

The number denotes the number of the investigation on the specimen.

The results are plotted on graph 10.1 which compares the rate of accumulation of the different fibres within the lung. This graph has arithmetic scales or axes as the process of dust accumulation, at least within the period of these experiments, has been shown to be approximately proportional to the dose[6,9]. These data showed a coefficient of correlation of 0,60 between the lung load and the period of exposure. The regression line fitted to the points was extrapolated to estimate the fibres in the lungs of a baboon after 34 months (the full exposure period) and provide an additional data point on the main clearance graph (Fig. 10.1.2).

The extrapolation to 34 months was made on the assumption that the daily accumulation of dust was linear with respect to time.[6,9] This would seem to be a reasonable assumption bearing in mind the nature of the breathing process and the state of the atmosphere being inhaled.

10.1.2 Clearance Study

The results of the clearance study are set out in Table 10.1.2. Table 10.1.2 is similar to table 10.1.1 except that it records the data collected

during the survival period of the baboons, i.e. the time after they were removed from the exposure chamber until they were killed or died naturally.

In this study a detailed examination was made of one specimen (409) to estimate the variation likely to occur when counting fibres from different samples of the same lung. The estimates showed a standard error of 138 (16,3%) about the mean 845.

A similar smaller exercize was carried out on sample 410. In this case the standard error was 1,2 (15%) about a mean of 8.

In one case (487) the baboon had only been exposed for 21 months. This was extrapolated to 34 months (the full exposure period) to allow inclusion of this result with the results from the baboons which had been exposed for 34 months. The extrapolation was made using the linear relationship between period of exposure and lung load which had been established in the "build-up" studies.

The data points in this study were plotted on semi log axes because the clearance processes of the lung are generally considered to follow a negative exponential law[5]. Such laws plot as straight lines

on semi log paper. Also the log scale was necessary to accommodate the extreme range of the fibre load readings (0,26 - 845 Mf/g).

The graph (Fig. 10.2) showed a good correlation between the clearance of the fibres and the time of survival (r^2 = 0,82). Expressed in terms of concentration decay the clearance of glass fibre from the lung had a half life of 6 months.

Table 10.1.2 Clearance Study : Glass Fibre

Baboon No	Exposure (Months)	Survival (Months)	D:W Ratio	Mf/g* Wet	Mf/g* Dry	Mean Dry	Notes
402	34	12	0,07	5	74	74	Random
403	34	84	-	-	-	-	Still alive
409	34	6	0,14	81	579		RU1
			0,14	74	529		RU2
			0,14	13	93		RU3
			0,13	135	1038		RL1
			0,13	102	785		RL2
			0,15	135	900		LU1
			0,15	142	947		LU2
			0,12	142	1183		LL1
			0,12	96	800	845	LL2
410	34	30	0,20	1,5	7		RU
			0,22	2,0	9		RL
			0,23	1,4	6		LU
			0,22	2,4	11	8	LL
411	34	46	0,07	0,08	0,08	1,16	Random
486	34	82	0,10	0,03	0,26	0,26	Random
487	21	24	0,09	14,5	161	260+	21 months exposure

* Mf/g = millions of fibres/g dry tissue

+ 161 Mf/g at 21 months estimated equivalent to 260 Mf/g at 34 months.

10.2 Crocidolite

10.2.1 Build-up study

Notwithstanding the high hazard generally attributed to the inhalation of crocidolite[43] asbestos only one animal died during the period of exposure. It was therefore not possible to monitor the build-up of fibre in the lung as was done in the case of glass fibre (10.1.1).

10.2.2 Clearance Study

The data for the clearance study are set out in Table 10.2.2. In one case (375) the baboon had only been exposed for 21 months. This was extrapolated to 35 months using the linear relationship between fibre load and exposure found in the other build-up studies The notes following Table 10.1.1 apply to this table.

Table 10.2.2 Clearance Study Crocidolite

Baboon No	Exposure (Months)	Survival (Months)	D:W Ratio	Mg/g Wet	Mf/g Dry	Notes
364	35	35	0,13	1355	10422	Random
366	35	61	0,15	888	5756	Random
367	35	96				Still alive
369	35	42	0,15	1568	10541	Random
370	35	40	0,15	489	3260	
372	21	23	0,14	439	3136	
373	35	7 days		-	-	Sick, tissue not available
374	35	58	0,14	555	4114	Random
375	21	0	0,14	1007	11982*	
376	35	23	0,16	2298	14364	

* 7982 Mf/g at 21 months estimated equivalent to 11982 Mf/g at 35 months

The results of this clearance study are plotted in graph 10.2, the clearance graph. Only a moderate correlation between the lung load and period of survival was found ($r = 0,43$) but this may be partially related to the extremely low slope (-0,006) of the regression as the points are closely distributed about the regression line.

10.3 Amosite

The data relating to the amosite exposure are set out in Tables 10.3.1 and 10.3.2. The survival times are generally shorter than those for glass fibre and crocidolite as the experiment was undertaken much later and some of the exposed animals are still alive. Where fibre counts are quoted from

animals marked "Still Alive" the material was obtained by biopsy. As explained below this material was not, in fact, used in the study.

10.3.1 Build-up Study

As 3 animals died during exposure and an unexposed control was available it was possible to make a limited study of the fibre build up.

These results are plotted on graph 10.1, the lung retention graph. A good significant correlation (r = 0,99) between lung fibre load and time of exposure is demonstrated.

As in the case of crocidolite the notes following the section on the glass fibre exposure (10.1.1) apply to this table except that it was not necessary to provide a column for the mean of the readings as only one estimation was made on each sample.

Table 10.3.1 Build-up study : Amosite

Baboon No	Exposure (Months)	Survival (Months)	D:W Ratio	Mf/g Wet	Mf/g Dry	Notes
5	15	0	0,16	719	4491	Random
9	41	0	0,14	945	6750	Random
10	46	0	0,18	2601	14467	Random
488	0	0	0,15	0,01	0,07	Random

animals marked "Still Alive" the material was obtained by biopsy. As explained below this material was not, in fact, used in the study.

10.3.1 Build-up Study

As 3 animals died during exposure and an unexposed control was available it was possible to make a limited study of the fibre build up.

These results are plotted on graph 10.1, the lung retention graph. A good significant correlation (r = 0,99) between lung fibre load and time of exposure is demonstrated.

As in the case of crocidolite the notes following the section on the glass fibre exposure (10.1.1) apply to this table except that it was not necessary to provide a column for the mean of the readings as only one estimation was made on each sample.

Table 10.3.1 Build-up study : Amosite

Ba[illegible]on No	Exposure (Months)	Survival (Months)	D:W Ratio	Mf/g Wet	Mf/g Dry	Notes
5	15	0	[illegible]16	719	4491	Random
9	41	0	0,14	945	6750	Random
10	46	0	0,18	2601	14457	Random
488	0	0	0,15	0,01	0,07	Random

10.3.2. Clearance Study

The data available for this study set out in Table 10.3.2. It will be seen that five of the animals which survived the exposure are still alive and their lung tissue was not available in the required quantities. In two cases biopsies had been taken early in the exposure (14 mnths) but in view of the small amount of tissue available and the long period between the biopsy and the end of the exposure it was not considered legitimate to extrapolate these results to the full exposure period (49 mnths).

The available data has been plotted on graph 10.2 the lung clearance graph. Since the exposure to amosite took place after the exposure to the other fibres the period of survival has been for shorter that that of the other fibres. However a steady clearance can be observed. The half life of the clearance appears to be about 18 months.

Table 10.3.2 Clearance Study : Amosite

Baboon No	Exposure (Months)	Survival (Months)	D:W Ratio	Mf/g Wet	Mf/g Dry	Mean Dry	Notes
1	49	33	-				Still alive
2	49	33	-				Still alive
3	49	25	0,19	576	3032		Random
			0,19	375	1974	2503	Random
4	49	33					Still alive
6	49	6	0,15	1570	10467	10467	Random
7	49	33					Still alive
8	49	25	0,18	1277	7094	7094	Random
11	14*	33	0,18	1702	9616	9616	Still alive
12	14*	33	0,25	425	1700		Still alive
989	49	6	0,18	7567	42039		Random
1096	49	6	0,16	13433	83956		Random

*Biopsy

10.4 Chrysotile

Only one animal survived the chrysotile exposure (See Fig. 3.3.4). The reason for the heavy mortality during this exposure was never established but it is the complete reverse of human experience. This suggests that chrysotile is the least harmful of all the asbestos types while crocidolite is considered the most harmful.[43]

10.4.1. Build-up study

By studying the fibre loads in the lungs of baboons which died at different times during the exposure period it was possible to determine the rate at which the fibre load built up during exposure. This data is set out in Table 10.4.1.

Table 10.4.1 Build-up study : Chrysotile

Baboon No	Exposure (Months)	Survival (Months)	D:W Ratio	Mf/g Wet	Mf/g Dry	Notes
13	14	0	0,13	18	138	
14	12	0	0,07	7,0	106	
15	21	0	0,09	2,6	28,4	
16	19	0	0,06	5,0	80,7	
17	26	0	0,10	3,4	33,7	
18	10	0	0,09	2,0	22,4	
19	21	0	0,09	7,8	86,7	
20	26	33				Still alive
21	26	0	0,12	11,3	139	
22	7	0	0,11	5,5	50,4	
23	13	0	0,08	4,9	61	
24	30	0	0,14	12	84	

These data are plotted on graph 10.1. From this graph it can be seen that there is only a low ($r = 0,2$) correlation between the lung fibre load (Mf/g dry tissue) and the length of exposure. Although there appeared to be a slight increase with time it was not significant.

A better correlation ($r = 0,62$) was obtained by plotting the logarithm of the lung load against the time of exposure. (Fig. 10.4.2.) This indicates that the rate of increase in the lung load is at least partially proportional to the existing fibre load in the lung and not only to the volume of air (or number of fibres) inhaled. Such an exponential increase could come about if the previously inhaled

fibre split into finer fibrils in the lung. This possibility was briefly investigated by taking a Scanning Electron Micrograph of fibres extracted from the lung of a baboon (Fig. 10.4.3). This should be compared with a similar photograph of the airborne fibres before inhalation (Fig. 3.1.1.2). Disintegration and splitting of the fibres is very apparent.

10.4.2. Clearance Study

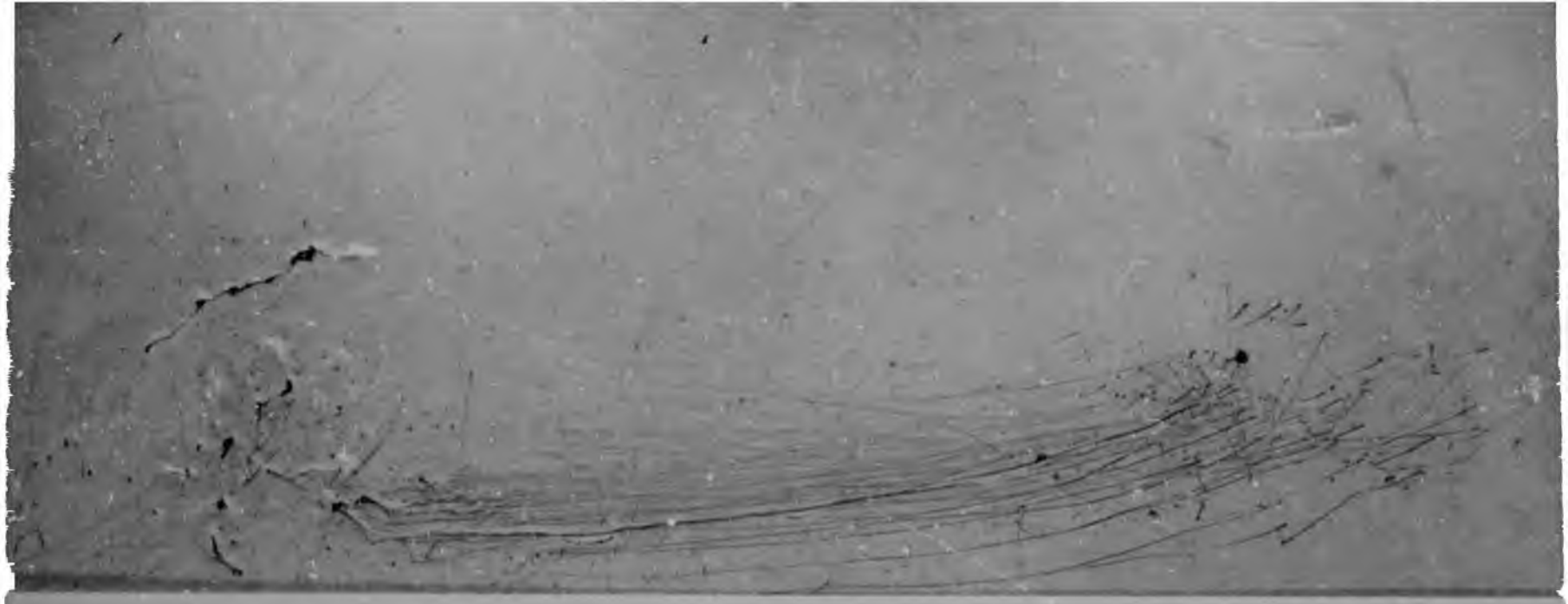
Unfortunately no material was available for the study of fibre clearance due to the high mortality of the animals during exposure.

10.5 Summary

These studies have shown that glass fibre builds up in the lung more slowly than the amphibole fibres and is cleared more quickly. Chrysotile also builds up more slowly that the amphiboles but the lung load seems to be enhanced by the inhaled fibres splitting into finer fibrils during residence in the lung.

The clearance half lives of the different fibres were shown to be as follows:

Glass fibre	6,4 months
Crocidolite	50 months
Amosite	18 months
Chrysotile	No material available



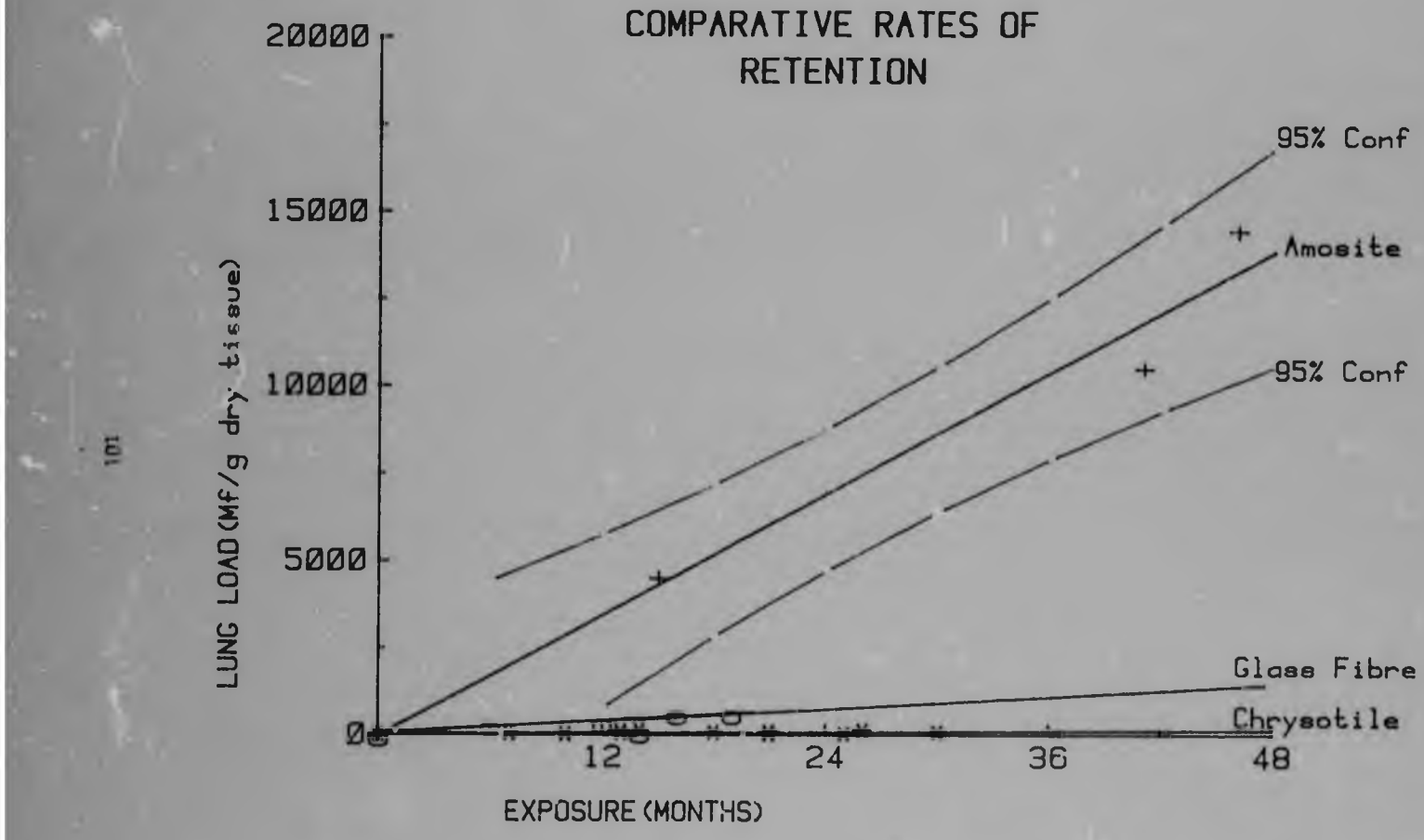

COMPARATIVE RATES OF
RETENTION
20000
15000
10000
5000
0
LUNG LOAD (Mf/g dry tissue)
95% Conf
Amosite
95% Conf
Glass Fibre
Chrysotile
12
24
36
48
EXPOSURE (MONTHS)

FIG 10.1

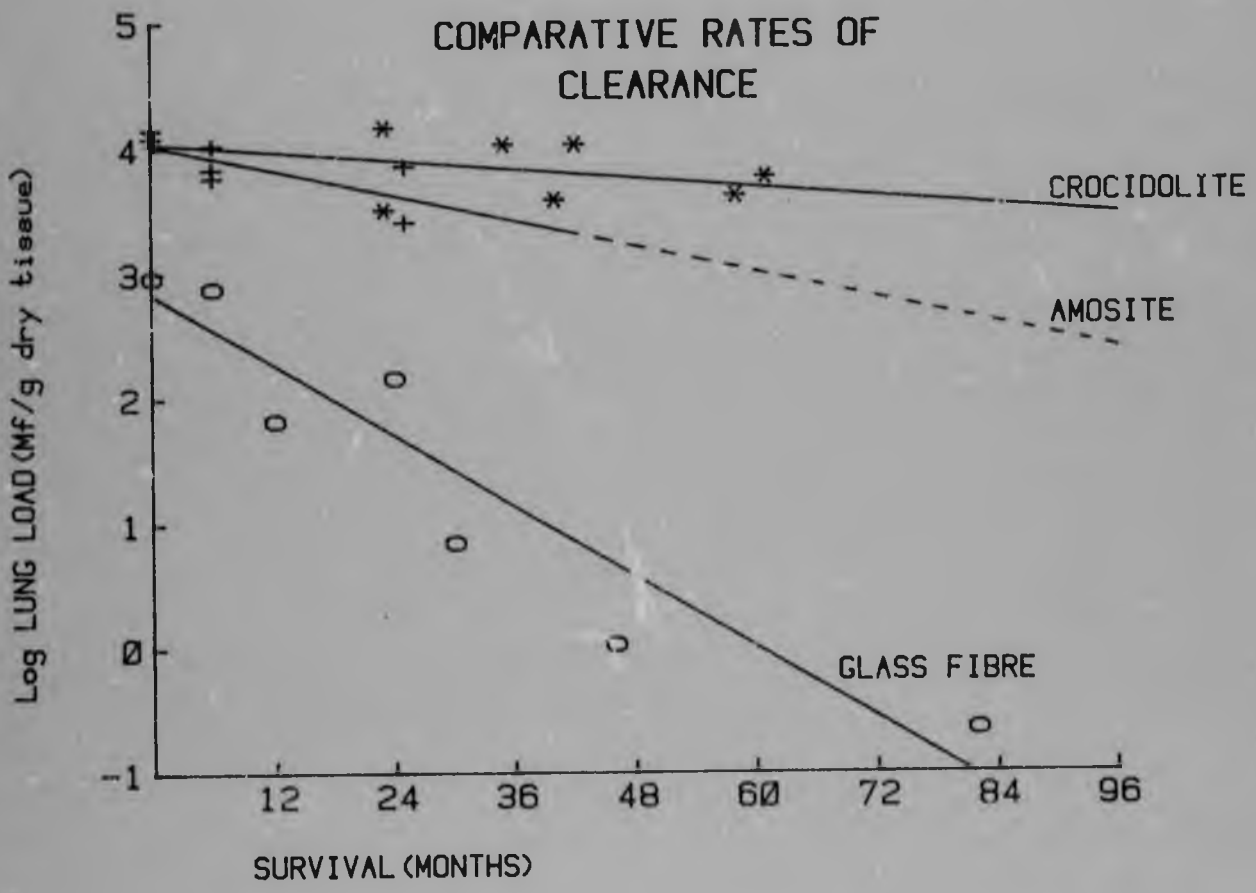

COMPARATIVE RATES OF CLEARANCE
Log LUNG LOAD (Mf/g dry tissue)
5
4
3
2
1
0
-1
12
24
36
48
60
72
84
96
SURVIVAL (MONTHS)
CROCIDOLITE
AMOSITE
GLASS FIBRE

FIG 10.2

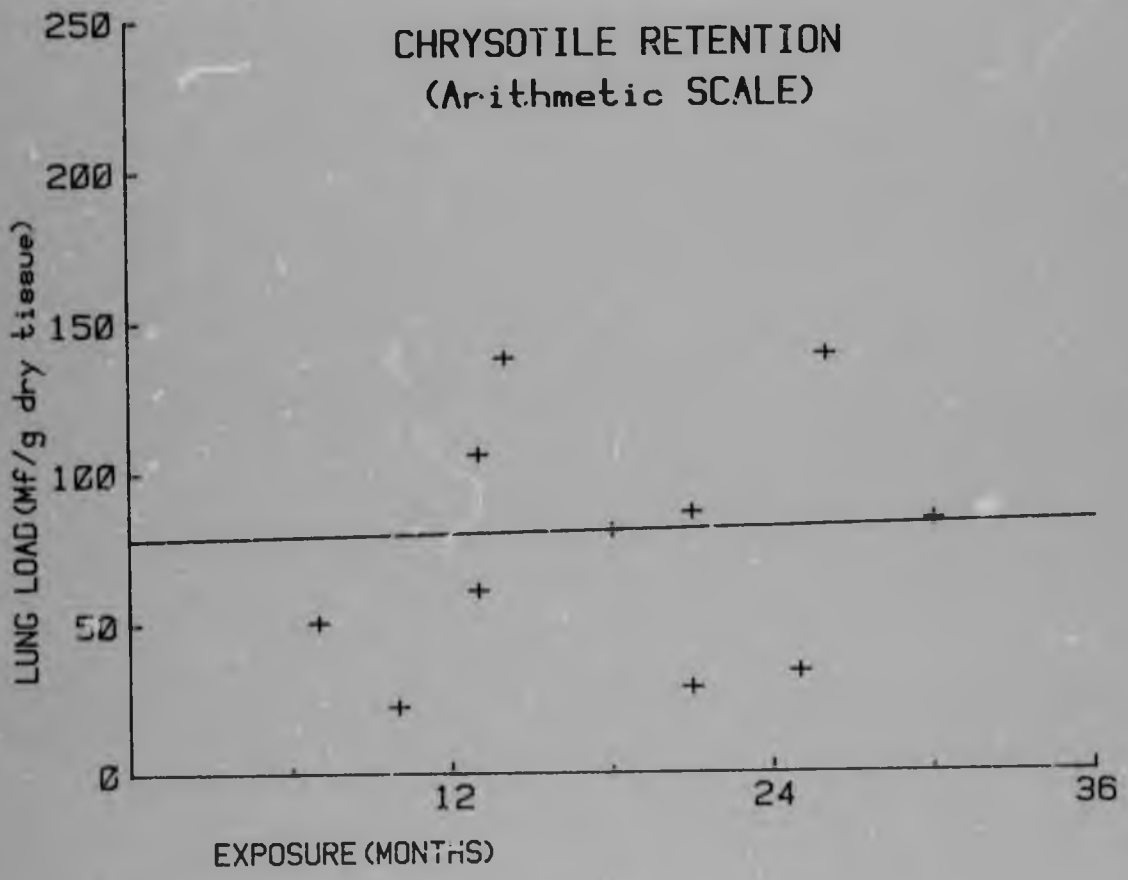

CHRYSOTILE RETENTION
(Arithmetic SCALE)
LUNG LOAD (Mf/g dry tissue)
250
200
150
100
50
0
12
24
36
EXPOSURE (MONTHS)

FIG 10.4.1

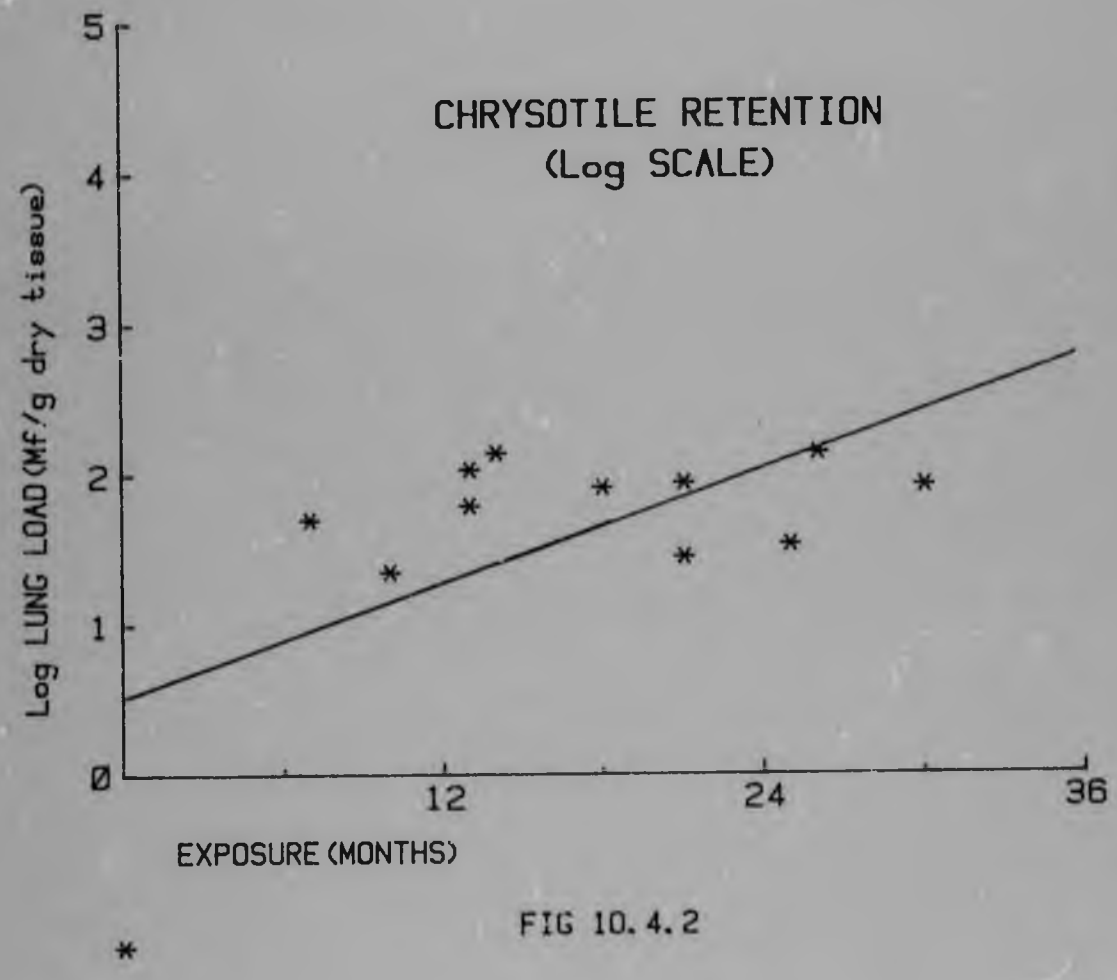

CHRYSOTILE RETENTION
(Log SCALE)
Log LUNG LOAD (Mf/g dry tissue)
0
1
2
3
4
5
12
24
36
EXPOSURE (MONTHS)

FIG 10.4.2

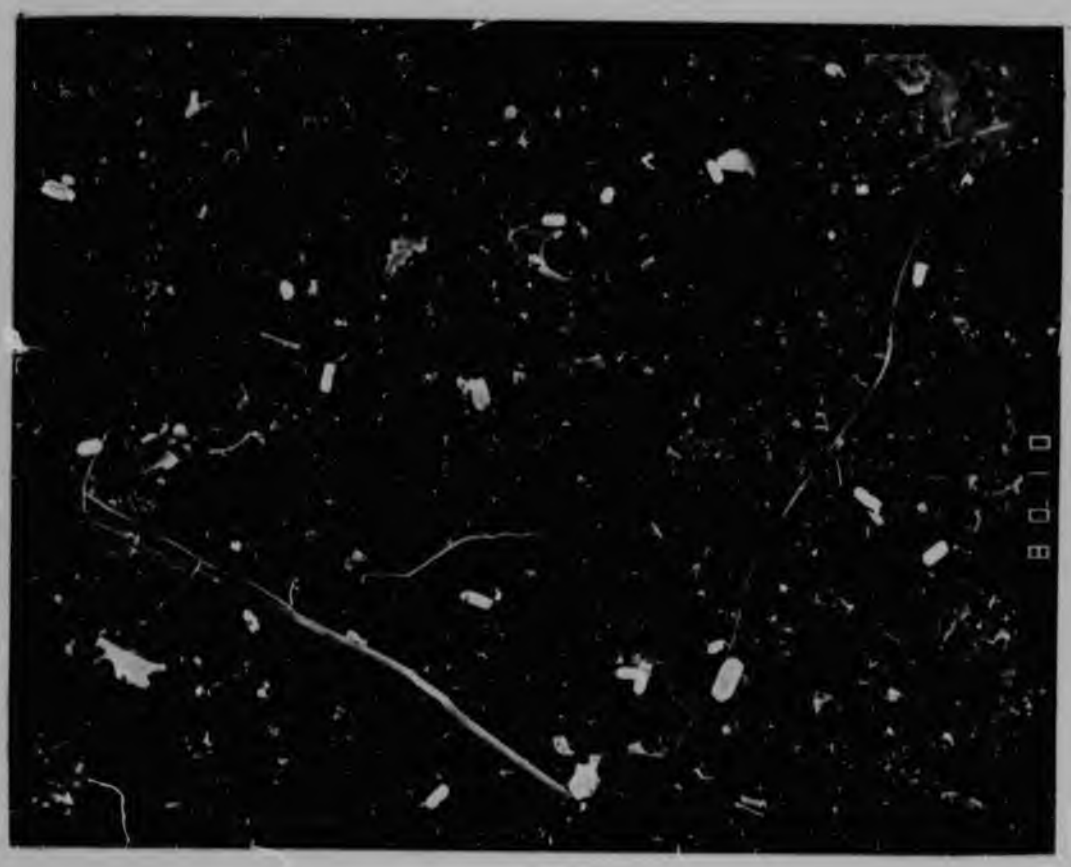


FIG 10.4.3 Chrysotile fibres extracted from lung of baboon showing splitting.

CHAPTER 11

STATISTICS

The statistics covered in this chapter are mainly concerned with the confidence limits which should be applied to the various results obtained and the significance of the trends shown by the graphs. The chapter has been divided into two main parts and a summary as follows.

11.1 Dust inhalation, in which the exposures to the different types of fibre are compared.

11.2 Retention of fibres in the lungs during exposure and the determination of the half lives or times of clearance of different types of fibre from the lung after cessation of exposure is discussed.

11.3 Summary

11.1 Dust inhalation

The most important part of this analysis was to determine if there were significant differences between the different dust exposures. Graphs of the dust concentrations v. days of exposure with fitted linear regressions were shown in Chapter 4 Figs. 4.3.1. - 4. Two regression lines were fitted to the crocidolite data to cover two distinct periods in that exposure i.e.

1. A period of 166 days in which the dust concentration was deliberately reduced to protect the animals health.
2. A period of 889 days in which the dust concentration was kept as constant as possible.

As far as the total dust dose of the animals was concerned this exposure is treated as one period of 1055 days.

The data related to the fibre concentrations in the different rooms was analysed using a one way analysis of variance (ANOVA). This compared the variance of the different airborne fibre samples within a group, in this case a dust room, with the overall variance between groups.

The ratio of the mean variance between groups (M_B) to the mean variance within groups (M_W) is generally referred to as F where $F = \frac{M_B}{M_W}$

Where the mean variation between groups is significantly greater than the mean variation within groups F will have a value greater than 1. The degree of significance of different values of F and different numbers of samples may be found in statistical tables[49]. A target concentration of 1000 fibres per cubic centimetre of air was aimed at but failure to achieve this was not important, it was only a guide line to the operator in charge of the rooms.

The first analysis of variance showed F = 6,58 indicating a significant difference between the dose of dust received by the animals in the different rooms. This was reduced to F = 0,20 by disregarding the chrysotile exposure in which none of the animals survived to the end. It may be concluded from

this analysis that the dust concentrations in the glass fibre, crocidolite and amosite rooms were not significantly different. However, it should be noted that the period of exposure in the amosite rooms (49 months) was longer than the exposure in the glass fibre and crocidolite rooms (35 months). It was considered this increase of 40% would not influence the rate of lung clearance materially as lung loads can vary by factors of hundreds or even thousands. The length of exposure in the chrysotile rooms was also 49 months but as only one animal survived this period and its lungs were not available for examination this was of no consequence.

The ANOVA did show that there was a significant difference between the exposures in the rooms and the target but this is not important. As stated previously the target was only an arbitrary figure given to the dust room operator as a guide. The ANOVA was carried out on an IBM AT PC using the program NANOSTAT from the London School of Hygiene and Tropical Medicine.

11.2 Fibre Retention and clearance

Before presenting the plotted results it is appropriate to discuss the degree of accuracy which may be expected from the determination of the lung fibre loads. This was investigated by taking 9 samples from a single lung (409) and analysing the values obtained.

The results (See Table 11.2) proved to be normally distributed with a mean of 852 fpcc and a standard error of 138 fpcc (16,3%). The basic statistics of these data are set out in Table 11.2.

Table 11.2 Statistics of Multiple fibre estimations on same lung

Statistic	Value	Comments
Number of values	9	
Mean	851	
Std Error of mean	138	
Std Deviation	415	
95% Conf. Interval	522-1170	
Skewness	- 0,29	= 0 when distribution is truly normal
Std mean dev	0,80	Distribution normal at 10% level of significance
Kurtosis	-	Too few samples to determine Kurtosis

11.2.1 Fibre Retention

The lung loads of animals which died during exposure were plotted against their time of exposure and a linear regression fitted. Three sets of points and three regression lines were plotted on the retention graph Fig. 10.1 (lung load v exposure time) to allow ready comparison of the rates of retention of the different fibres. The axes of the graph were scaled arithmetically to comply with the generally accepted assumption that lung load is proportional to inhaled dose.

Two of the sets of data, those relating to glass fibre and amosite, showed a linear increase in lung load with length of exposure which was significant at the 5% level. The significance was ascertained by comparing the correlation coefficient with the upper critical values for the appropriate number of points given in a table of product moment correlations upper critical values[49].

For the third regression the slope was not significant (n = 12, r = 0,185) but it was significant when the lung load was transformed into logarithms. A linear regression on semi log axes is equivalent to an exponential increase of lung load with time of exposure. This type of relationship implies that the lung load is at least partially proportional to the existing lung load. This could come about if the fibres in the lung gradually split into finer fibrils. Evidence of this effect is shown in the scanning electron micrograph, Fig. 10.4.3.

Although a significant difference in the slopes of the amosite regression and those of glass fibre and chrysotile can be predicted with some certainty from inspection of Fig. 11.2.1. it can be confirmed by calculating the 95% conf limits of the regression. This has been done and it can be seen that the amosite retention is significantly different from chrysotile and glass fibre.

Table 11.2.1 Fibre Build-up :Regressions

Fibre Type	n	Regression Eqn.	r	Remarks
Glass fibre	6	$\bar{y} = 23{,}047 + 30x$	0,77	Significant 5% level
Crocidolite	-	-		No data available
Amosite	4	$\bar{y} = -31{,}85 + 290x$	0,99	Significant 5% level
Chrysotile (Lin)	12	$\bar{y} = 32{,}58 + 2{,}2x$	0,43	Not significant at 5% level
Chrysotile (logs)	12	$\bar{y} = 0{,}51 + 0{,}064x$	0,62	Significant at 5% level

11.2.2 Fibre clearance

The data presented under this heading is the most important data of the whole work: it is the crux of the whole investigation: it is the comparison of the rates of clearance of the different types of fibre. Unfortunately only one animal survived the chrysotile exposure, so no data is available on the clearance of chrysotile fibres.

The available data has been plotted on Fig. 10.2. This is effectively a semi log graph with the logarithm of the lung load (y) plotted against the time of survival (x).

This scaling has been chosen to linearise the negative exponential curve of the lung clearance pattern.

From this graph it can be seen that the clearance of glass fibre is significantly different from crocidolite and amosite. The crocidolite and amosite were shown not to be significantly different from each other by calculating the 95% confidence limits of the crocidolite regression.

The upper confidence limit of this regression leads one to the conclusion that the clearance of crocidolite is virtually non-existent, at least under the conditions used in these experiments.

The half lives of the clearance from the lung was calculated by dividing the slope of the regressions into log 0,5 (-0,3010). This gave the time in months for the lung load to fall to one half it present value. The values for the different fibres are set out in Table 11.2.2.

Table 11.2.2 Fibre Clearance : Regressions and Half Lives

Fibre type	n	Regression eqn	n	Half life
Glass fibre	7	$\hat{y} = 2,817 - 0,047\ x$	0,93	$\frac{-0,301}{-0,047} = 6,4$ mnths
Crocidolite	8	$\hat{y} = 4,038 - 0,006\ x$	0,43	$\frac{-0,301}{-0,006} = 50,2$ mnths
Amosite	6	$\hat{y} = 4,025 - 0,017\ x$	0,71	$\frac{-0,301}{-0,017} = 17,7$ mnths
Chrysotile	-	-		No data available

11.3 Summary

The statistical analysis of the data collected in this study was carried out in two parts.

1. The conditions in the dusting rooms
2. The retention and clearance of inhaled fibres.

The first analysis showed that the concentration of fibres in the rooms containing glass fibre, crocidolite and amosite were not significantly different. The concentration in the fourth room, chrysotile was different from the others but since only one animal survived the exposure in this room this did not influence the results.

In the second part of the analysis the rate of accumulation of fibres in the lungs was shown to be far faster for amosite than for glass fibre or chrysotile. No figures were available for crocidolite since no animals died during the crocidolite exposure.

Glass fibre was shown to clear from the lungs far faster than crocidolite and amosite. No figures were available for the clearance of chrysotile since only one animal survived the exposure and its lung was not available for examination.

These two paragraphs, when considered together might explain much of the enhanced reaction of the lungs to amphibole asbestos.

CHAPTER 12

DISCUSSION

This work has shown the difference between the retention and clearance of amphibole asbestos fibres and glass fibres and chrysotile more clearly than any other work with which the author is familiar. It has long been assumed that glass fibre and chrysotile asbestos do not enter the lung as freely as the amphibole fibres but the degree of this difference has not been established. Similarly it has been assumed that glass fibre and chrysotile are cleared more rapidly than the amphiboles but once again there has been no measurement of the difference. That there should be differences in the treatment of these similar materials by the lung might be inferred from their microscopic characteristics. Although they are all made up of fibres they are very different fibres. Amphiboles are very fine and virtually indestructible while chrysotile, though fine, is soft and fluffy and is liable to degradation within the lung. Glass fibre differs from all types of asbestos in as much as it breaks across its diameter rather than splitting down its length which is a common feature of asbestos.

In this investigation the differences between the retention of amphibole fibres and the other fibres, glass fibres and chrysotile have been shown to be very great, far greater than the statistical variation in the number of fibres found in portions cut from the same lung. Clearly the samples cut from lungs exposed to different fibres came from significantly different fibre concentrations. It was only because the differences in the fibre loads in the lungs which had been exposed to the amphibole fibre and those which had been exposed to glass fibre and chrysotile were so great that it was possible to detect them in the material used.

This material came from animals which had been exposed to fibres to investigate the pathological and immunological effects of the exposure not the rates of retention and clearance. It was only when animals died that their lungs became available for examination. This meant that the lung samples were not spaced ideally to investigate either the gradual build up of fibre load with length of exposure or the gradual decline during the survival period. Additionally no material was available to study the build up of crocidolite in the lung or the clearance of chrysotile. This last is a serious deficiency in the study. The comparison of the clearance of chrysotile with the amphiboles would have been a valuable outcome from the investigation.

Notwithstanding these lapses from perfection the material available provided a unique base from which the different rates of fibre retention and clearance by the lungs of human workers could be predicted.

Firstly the lungs of the baboons are very similar to human lungs. Secondly, the concentrations of the fibres inhaled had been measured and thirdly the length of exposure and survival after exposure was accurately recorded.

It is difficult to determine the accuracy of the fibre counts used to determine the concentration of the airborne fibres inhaled and the fibre loads in the lungs. However this may be gauged from the results of an international counting trial in which the NCOH microscopist participated. The results of this trial which referred to airborne

fibres are presented in Fig. 12.1. The reference number of this laboratory was 32. From the diagram it can be seen that for low density slides the NCOH counts tended to be high but for high density slides the mean was straddled. From these results it may be assumed that the NCOH counting is no better or no worse than that in other major laboratories throughout the world. A similar trial comparing estimates of fibres in lung tissue has been conducted but the results of this are not available at the time of writing.

A graph, Appendix D showing the clearance of fibres from the lungs of crocidolite miners is included for comparison. In preparing this graph the asbestos exposure was not known as accurately as the exposure of the baboons. It was estimated from the man's work history and mean fibre concentrations of the type of work he had done. These latter figures had been determined by Inspectors of the Department of Mineral and Energy Affairs over many years.

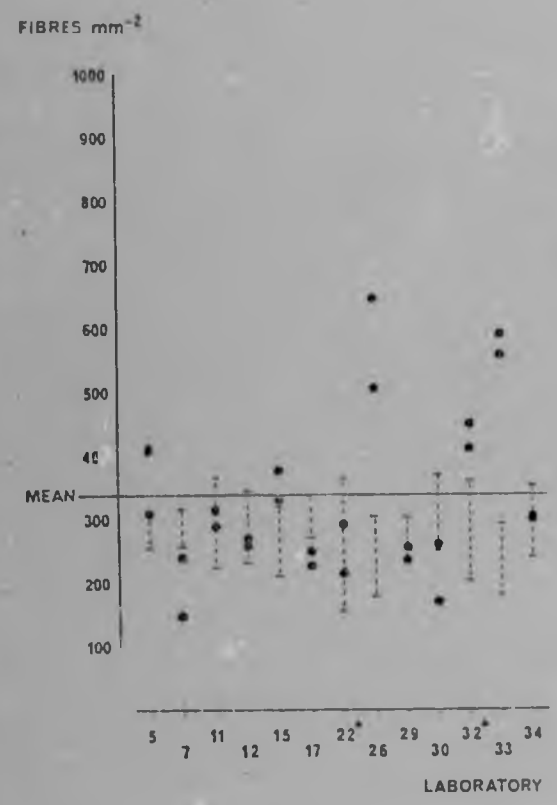
Because of the different exposures of the miners as opposed to the equal exposure of the baboons it was necessary to plot the ratio lung fibre load at death/estimated fibre dose in life against period of survival to determine the rate of clearance of fibres. If this ratio decreased with time it would indicate that clearance was taking place. A typical graph plotted in this way is included as Appendix D.

Although the miners' exposure was not as well established as the baboons, many more data points were available: 34 instead of 6 or 7. The half life of the fibre clearance of the miners determined from this graph was 5,8 yrs (70 months) which compared well with the value of 50

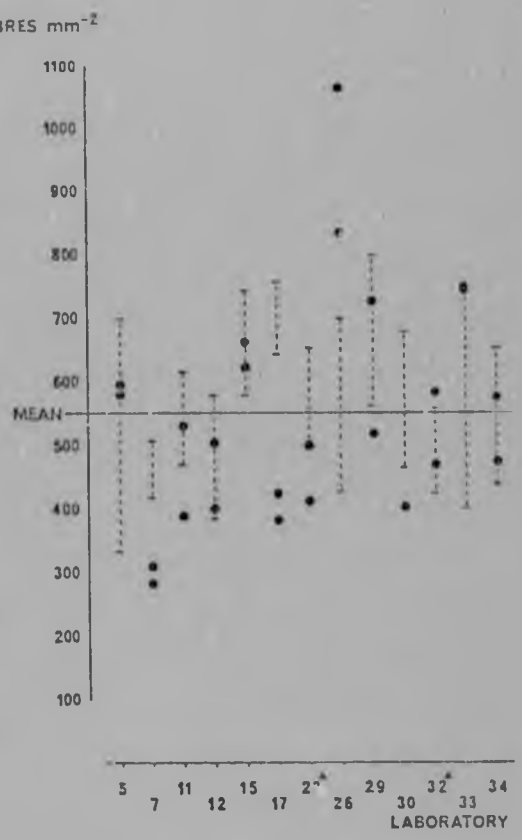
months predicted by the baboon model. To date it has not been possible to obtain material from humans who have been exposed to the other fibres, i.e. glass fibre, amosite and chrysotile to compare with the results obtained from the baboons but this work is envisaged for the future.

Conclusions

This work has shown that the retention of the amphibole fibres by the lung may proceed at ten times the rate of retention of glass fibre and chrysotile. Also the clearance of the amphibole is eight times slower than the clearance of the other fibres. These two observations may, in part at least, explain the more serious sequelae of the inhalation of amphiboles; crocidolite in particular.



'LOW' DENSITY



HIGH DENSITY

Light microscopy

▲ more than 100 fibres counted

T range of quality control counts

FIG 12.1 International counting trials
(NCOH = Laboratory 32)

REFERENCES

1. Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the north Western Cape Province. Br J Ind Med 1960; 17: 260-271.

2. Wright GW, Kuschner M. The influence of varying lengths of glass and asbestos fibres on tissue response in guinea pigs. In: Walton WH ed. Inhaled Particles IV, part 2. Oxford: Pergamon, 1977: 455-474.

3. Peto J, Seidman H, Selikoff IJ. Mesothelioma mortality in asbestos workers: implication for models of carcinogenesis and risk assessment. Br J Cancer 1982; 45: 124-135.

4. Goldstein B, Rendall REG, Webster I. A comparison of the effects of exposure of baboons to crocidolite and fibrous-glass dusts. Environ Res 1983; 32: 344-359.

5. Morgan A, Evans J G, Holmes A. Deposition and clearance of inhaled fibrous minerals in the rat. Studies using radioactive tracer techniques. In: Walton WH, ed. Inhaled Particles Iv, part 2. Oxford: Pergamon 1977: 259-274.

6. Bolton RE, Vincent JH, Jones AD, Addison J, Beckett ST. An overload hypothesis for pulmonary clearance of UICC amosite fibres inhaled by rats. Br J Ind Med 1983; 40: 264-272.

7. Vincent JH, Johnston AM, Jones AD, Bolton RE, Addison J. Kinetics of deposition and clearance of inhaled mineral dusts during chronic exposure. Br J Ind Med 1985; 42: 707-715.

8. The activities of the South African Committee on cumulative dust exposure records. Ann Occup Hyg 1977; 20: 271-277.

9. Wagner JC, Berry G, Skidmore JW, Timbrell V. The effects of the inhalation of asbestos in rats. Br J Cancer 1974; 29: 252-269.

10. Middleton AP, Beckett ST, Davis JMG study of the short-term retention and clearance of inhaled asbestos by rats, using UICC standard reference samples. In: Walton WH, ed. Inhaled Particled IV, part 1. Oxford: Pergamon, 1977: 247-258.

11. Middleton AP, Beckett ST, Davis JMG. Further observation on the short-term retention and clearance of asbestos by rats, using UICC reference samples. Ann Occup Hyg 1979; 22: 141-152.

12. Hounan RF. The Konimiser - a dispenser for the continuous generation of dust clouds from milligram quantities of asbestos. Ann Occup Hyg 1971; 14: 329-335.

13. Morgan A, Talbot RJ, Holmes A. Significance of fibre length in the clearance of asbestos fibres from the lung. Br J Ind Med 1978; 35: 146-153.

14. Morgan A, Holmes A, Davison W Clearance of sized glass fibres from the rat lung and their solubility in vivo. Ann Occup Hyg 1982; 25: 317-331.

15. Pritchard JN, Holmes A, Evans JC, Evans N, Evans RJ, Morgan A. The distribution of dust in the rat lung following administration by inhalation and by single intratracheal instillation. Environ Res 1985; 36: 268-297.

16. Roggly VL. Brody AR. Changes in numbers and dimensions of chrysotile asbestos fibers in lungs of rats following short-term exposure. Exp Lung Res 1983; 7: 133-147.

17. Bohning DE, Atkins HL, Cohn SH. Long-term particle clearance in man: normal and impaired. Ann Occup Hyg 1982; 26: 259-271.

18. Geographical Pathology Committee of the International Union against Cancer. Report and recommendations of the working group on asbestos and cancer. Br J Ind Med 1965; 22: 165-171.

19. Timbrell V, Rendall REG. Preparation of the UICC standard reference samples of asbestos. Powder Technol 1971/72; 5: 279-287.

20. Rendall REG. Data sheet on the chemical and physical properties of the U.I.C.C. standard reference samples. In: Shapiro HA, ed. Pneumoconiosis: proceedings of the International Conference Johannesburg 1969. Cape Town: Oxford University Press, 1970: 23-27.

21. Timbrell V. Characteristics of the International Union against Cancer standard reference samples of asbestos. In: Shapiro HA, ed. Pneumoconiosis: proceedings of the International Conference Johannesburg 1969 Cape Town: Oxford University Press, 1970: 28-36.

22. Timbrell V, Griffiths DM, Pooley FD. Possible biological importance of fibre diameters of South African amphiboles. Nature 1971; 232: 55-56.

23. Deer WA, Howie RA, Zussmann J. Rock-Forming Minerals. Vol. 2. Chain Silicates. London: Longmans, 1962: 203-204.

24. Deer WA, Howie RA, Zussmann J. Rock-Forming Minerals. Vol. 3. Sheet Silicates. London: Longmans, 1962: 171.

25. Goldstein B, Webster I, Rendall REG, Skikne MI. The effect of asbestos-cement dust inhalation on baboons. Eviron Res 1978; 16: 216-225.

26. American Conference of Governmental Industrial Hygienists. Air Sampling Instruments. 6th ed. Cincinnati: ACGIH, 1983.

27. Ashcroft T, Heppleston AG. The optical and electron microscopic determination of pulmonary asbestos fibre concentration and its relation to the human pathological reaction. J. Clin Path 1972; 26: 224-234.

28. Churg A, Sakoda N, Warnock ML. A simple method for preparing ferruginous bodies for electron microscopic examination. Am J Clin Path 1977; 68: 513-517.

29. Churg A, Warnock ML. Asbestos fibres in the general population. Am Rev Respir Dis 1980; 122: 669-678.

30. Carter RE, Taylor WF. Identification of a particular amphibole asbestos fiber in tissues of persons exposed to a high oral intake of the mineral. Environ Res 1980; 21: 85-93.

31. Berkley C, Churgh J, Selikoff IJ et al. The detection and localization of fibres in tissue. Ann NY Acad Sci 1965; 132: 48-63.

32. Chatfield EJ, Dillon MJ. Some aspects of specimen preparation and limitations of particulate analysis by scanning electron microscopy. Scan Electron Microsc 1979; 1: 563-579.

33. Lillie RD. Histopathologic Technic and Practical Histochemistry. 3rd ed, New York: McGraw-Hill, 1965: 651.

34. Keenan RG, Lynch JR. Techniques for the detection, identification and analysis of fibres. Am Ind Hyg Assoc J 1970, 31: 587-597.

35. Asbestos International Association. Reference Method for the Determination of Airborne Asbestos Fibre Concentrations at Workplaces by Light Microscopy (Membrane Filter Method) London: AIA, 1979.

27. Ashcroft T, Heppleston AG. The optical and electron microscopic determination of pulmonary asbestos fibre concentration and its relation to the human pathological reaction. J. Clin Path 1972; 26: 224-234.

28. Churg A, Sakoda N, Warnock ML. A simple method for preparing ferruginous bodies for electron microscopic examination. Am J Clin Path 1977; 68: 513-517.

29. Churg A, Warnock ML. Asbestos fibres in the general population. Am Rev Respir Dis 1980; 122: 669-678.

30. Carter RE, Taylor WT. Identification of a particular amphibole asbestos fiber in tissues of persons exposed to a high oral intake of the mineral. Environ Res 1980; 21: 85-93.

31. Berkley C, Churgh J, Selikoff IJ et al. The detection and localization of fibres in tissue. Ann NY Acad Sci 1965; 132: 48-63.

32. Chatfield EJ, Dillon MJ. Some aspects of specimen preparation and limitations of particulate analysis by scanning electron microscopy. Scan Electron Microsc 1979; 1: 563-579.

33. Lillie RD. Histopathologic Technic and Practical Histochemistry. 3rd ed, New York: McGraw-Hill, 1965: 651.

34. Keenan RG, Lynch JR. Techniques for the detection, identification and analysis of fibres. Am Ind Hyg Assoc J 1970, 31: 587-597.

35. Asbestos International Association. Reference Method for the Determination of Airborne Asbestos Fibre Concentrations at Workplaces by Light Microscopy (Membrane Filter Method) London: AIA, 1979.

36. Edwards GH, Lynch JR. The method used by the U.S. Public Health Service for enumeration of asbestos dust on membrane filters. Ann Occup Hyg 1968; 11: 1-6.

37. Rendall REG, Schoeman JJ. A membrane filter technique for glass fibres. Ann Occup Hyg 1985; 29: 101-108.

38. Haselmann H. One hundredth anniversary of Abbe's apochromats. Proc R Microsc Soc 1986; 21: 197-202.

39. Rooker SJ, Vaughan NP, LeGuen JM. On the visibility of fibers by phase contrast microscopy. Am Ind Hyg Assoc J 1982; 43: 505-515.

40. Walton WH, Beckett ST. A microscope eyepiece graticule for the evaluation of fibrous dusts. Ann Occup Hyg 1977; 20: 19-23.

41. Middleton AP. Visibility of fine fibres of asbestos during routine electron microscopical analysis. Ann Occup Hyg 1982; 25: 53-62.

42. LeGuen JM. Test slides for phase contrast microscopy. In: Proceedings of the Third Colloquium on Dust Measuring Technique and Strategy. Cannes (France) - June 10-12. 1980. London: Asbestos International Association, 1980: 83-96.

43. Schneider T. The influence of counting rules on the number and size distribution of fibres. Ann Occup Hyg 1979; 21: 341-350.

44. Great Britain. Health and Safety Commission. Asbestos. Vol. 1: Final Report of the Advisory Committee. London: HMSO, 1979: 52.

45. Beckett ST, Attfield MD. Interlaboratory comparisons of the counting of asbestos fibres samples on membrane filters. Ann Occup Hyg 1974; 17: 85-96.

46. Gibbs GW, Baron P. Beckett ST et al. A summary of asbestos counting experience in seven countries. Ann Occ Hyg 1977; 20: 321-332.

47. Hoyes JR, Clayton R. A routine for the control of the performance of microscopists evaluating airborne asbestos fibre samples on membrane filters by phase contrast microscopy. Ann Occup Hyg 1980; 23: 381-401.

48. Lilienfeld P, Elterman PB, Baron P. Development of a prototype fibrous aerosol monitor. Am Ind Hyg Ass 1979; 40: 270-282.

49. Stoker DJ. Statistical Tables 3rd Ed. Pretoria: Academia, [illegible] 14.

50. Morgan A, Holmes A. Distribution and characteristics of amphibole asbestos in left lung of an insulation worker. Br J Ind Med 1983; 40: 45-50.

51. Asbestos International Association. Method for the determination of airborne asbestos fibres and other inorganic fibres by scanning electron microscopy. LONDON : AIA, 1984 (AIA) Health and Safety Publication Recommended Technical Method No. 2 (RTM2)

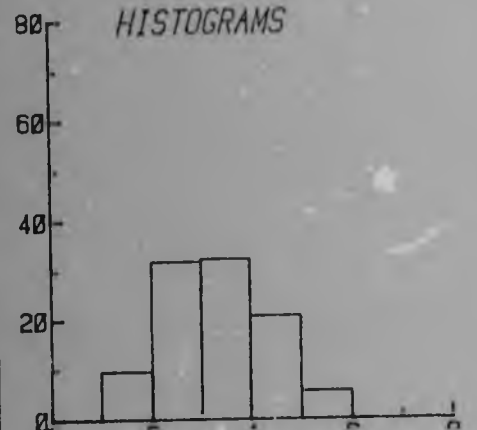
APPENDICES

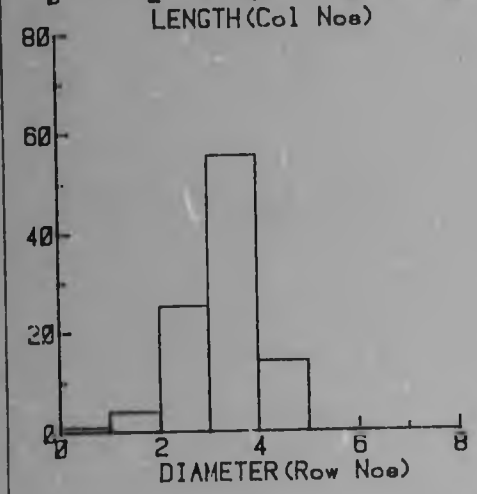
%SIZE DISTRIBUTION BY LENGTH AND DIAMETER

SAMPLE: TPG/FA RM6
N= 198

DATE: 30-8-87

HISTOGRAMS





DIAMETER (MICRONS)	1.25	2.5	5	10	20	40	80	160	% DIAM DISTRIB
.04			.51	.51					1.01
.08		1.01		2.02	1.01				4.04
.16		5.05	12.63	6.06	1.52				25.25
.32		3.54	14.14	20.71	14.65	2.53			55.56
.64			4.55	3.03	3.54	3.03			14.14
1.28									Ø
2.56									Ø
5.12									Ø
	Ø	9.6	31.82	32.32	20.71	5.56	Ø	Ø	

%LENGTH DISTRIBUTION

NOTES

Airborne GLASS FIBRE Room 6

APPENDIX A(1)

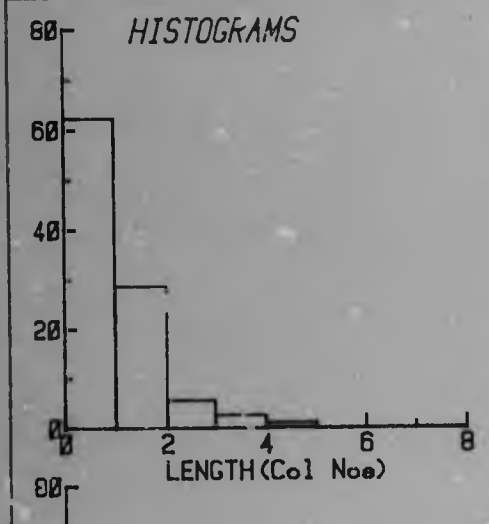
%SIZE DISTRIBUTION BY LENGTH AND DIAMETER

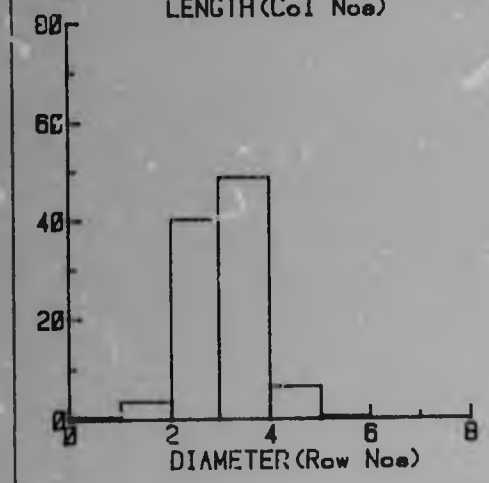
SAMPLE: TPC [illegible] (A)

DATE: 30-8-87

N= 199

HISTOGRAMS





DIAMETER (MICRONS)	LENGTH (MICRONS) 1.25	2.5	5	10	20	40	80	160	% DIAM DISTRIB
.04									
.08	.5								.5
.16	2.51	1.01							3.52
.32	28.14	9.05	2.51		.5				40.2
.64	28.64	15.58	1.51	2.51	.5				48.74
1.28	2.61	3.02	1.51						6.53
2.56	.5								.5
5.12									0
									0
	62.31	28.64	5.53	2.51	1.01	0	0	0	

%LENGTH DISTRIBUTION

NOTES

Airborne CROCIDOLITE Room 4

APPENDIX A (2)

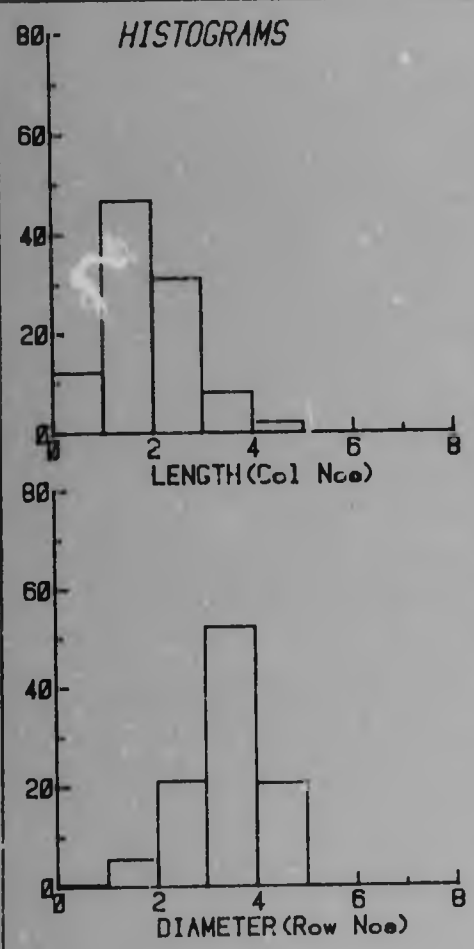
127

%SIZE DISTRIBUTION BY LENGTH AND DIAMETER

SAMPLE: AMO(A) RM3 DATE: 30-8-87

N= 199

HISTOGRAMS



DIAMETER (MICRONS)	LENGTH (MICRONS) 1.25	2.5	5	10	20	40	80	160	% DIAM DISTRIB
.04			.5						.5
.08	1.01	1.51	3.02						5.53
.16	2.01	10.05	6.53	1.51	1.01				21.11
.32	8.04	25.63	14.07	4.02	.5				52.26
.64	1.01	9.55	7.04	2.51	.5				20.6
1.28									0
2.56									0
5.12									0
	12.06	46.73	31.16	8.04	2.01	0	0	0	

%LENGTH DISTRIBUTION

NOTES

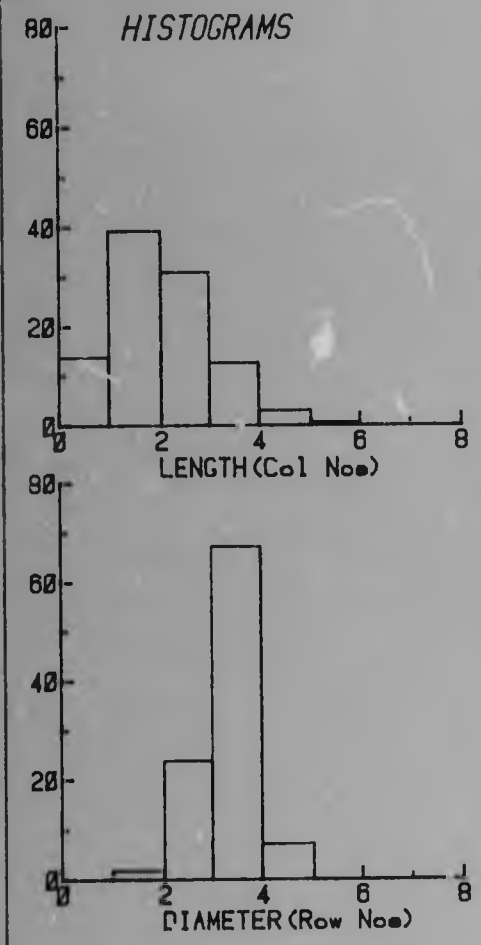
Airborne AMOSITE Room 3

%SIZE DISTRIBUTION BY LENGTH AND DIAMETER

SAMPLE: CHRYS RM6 DATE: 30-8-87

N= 168

HISTOGRAMS



DIAMETER (MICRONS)	1.25	2.5	5	10	20	40	80	160	% DIAM DISTRIB
.04									0
.08	1.19	.6							1.79
.16	3.57	9.52	7.14	2.38	1.19				23.81
.32	8.33	26.79	21.43	8.93	1.79				67.26
.64	.6	2.38	2.38	1.19		.6			7.14
1.28									0
2.56									0
5.12									0
	13.69	39.29	30.95	12.5	2.98	.6	0	0	

%LENGTH DISTRIBUTION

NOTES

Airborne CHRYSOTILE Room 6

APPENDIX A (4)

APPENDIX B

An alternative Detection Limit Test Slide is now commercially available, as follows: HSE/NPL TEST SLIDE (MARK II) FOR THE DETERMINATION OF DETECTION LIMIT WHEN USING PHASE CONTRAST MICROSCOPY.

Available from:

PTR OPTICS,
UNIT D9,
CROSS GREEN APPROACH,
CROSS GREEN INDUSTRIAL ESTATE,
LEEDS,
YORKSHIRE,
UNITED KINGDOM

DESCRIPTION

The standard test slides consist of identical epoxy replicas (with a refractive index of 1.58) of a Master Slide produced and certified by the National Physical Laboratory (U K) The epoxy replicas are mounted on a glass slide 75 x 25 x 1.2 or 75 x 25 x 0 8 mm and covered by a coverslip 0.17 mm thick with a layer of another resin with a refractive index 1 49 in between. The test objects consist of a series of seven blocks of grooves of length 8.5 mm filled with a resin of refractive index 1 49 in a medium of refractive index 1.58. The grooves have a V-shape profile and have a depth-to-width ratio of about 0.1. The blocks are separated by gaps 20 micrometres wide. A set of four deep marker grooves is placed on either side of the array and a further two sets of two marker grooves spaced at an interval of 120 micrometres intersect the array at right angles. The zone of the test objects to be used is delineated by the rectangle bounded by these marker grooves. This zone can easily be located, as the field of view in which it is found is engraved on the coverslip. This is illustrated in Figures 1 and 2.

The widths of the grooves within each block and the calculated phase change (in degrees) associated with the maximum path difference in the light rays passing through the test objects are in Table I.

METHOD OF USE

Set up the microscope for phase contrast microscopy as recommended for the membrane filter method.

Locate Block 1 (the coarsest set) of the test objects and move the slide to observe adjacent blocks. Determine the block of the finest grooves that can be seen. It is unlikely that all seven blocks of grooves will be detected using optical phase contrast techniques, even on the best research microscope. On the basis of present information, a satisfactory system will detect Block 5.

Full details are supplied with the slide.

1 January 1982

TABLE I

Widths of Test Objects and Calculated Maximum Phase Change Induced in Light Rays passing through Test Objects of HSE/NPL Test Slide

Block Number	Groove Width (micrometres)	Maximum Calculated Phase Change (in degrees) for light rays λ = 530 nanometres passing through test objects
1	1.08	6.6
2	0.77	4.7
3	0 64	3.9
4	0.53	3.2
5	0.44	2.7
6	0.36	2.2
7	0.25	1.5

HSE/NPL Test slide for phase contrast microscopy

Fig. 1 Test slide (76 x 25 mm)

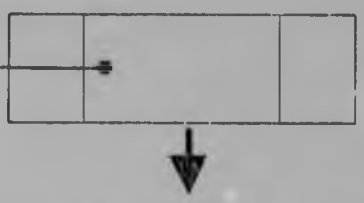
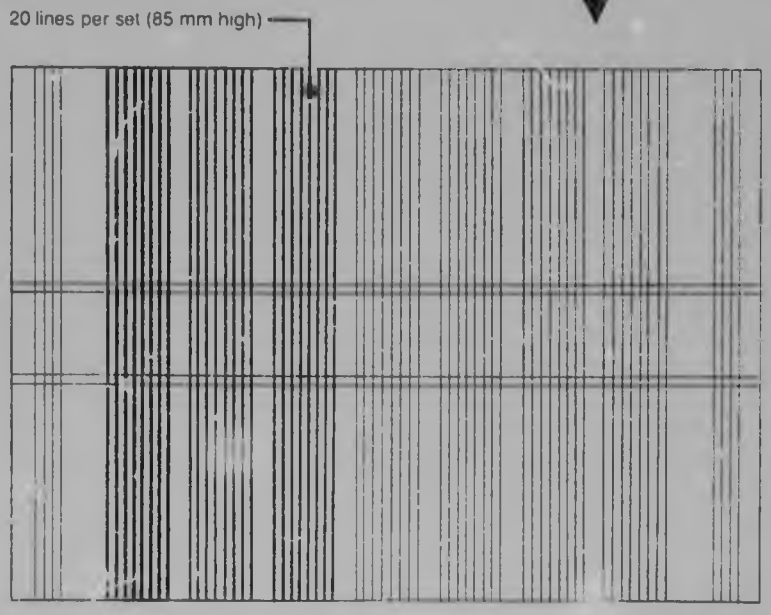


Fig 2 Enlarged field of view



APPENDIX C (1)

NATIONAL CENTRE FOR OCCUPATIONAL HEALTH

INDUSTRIAL HYGIENE DEPARTMENT

FIBRE COUNTING SHEET

PROJECT ..... FILTER NO. ..... DATE .....

INPUT DATA

GRATICULE AREA .....mm^2 VOL cc

FILTER DIAM .....mm^2 COUNT .....

COUNT

COUNT FIELDS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
25																					
24																					
23																					
22																					
21																					
20																					
19																					
18																					
17																					
16																					
15																					
14																					
13																					
12																					
11																					
10																					
9																					
8																					
7																					
6																					
5																					
4																					
3																					
2																					
1																					
COUNT	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
COL. TOTS																					

OUTPUT DATA

FIBRE CONC FIBRES/mm^2 .....

C.V. .....

95%	
fpcc	
95%	

REMARKS: .....

LIGHT MICROSCOPE FIBRE COUNTING SHEET.

.....

APPENDIX C(2)

NATIONAL CENTRE FOR OCCUPATIONAL HEALTH

INDUSTRIAL HYGIENE DEPARTMENT

FIBRE COUNTING SHEET

PROJECT ..... FILTER NO. ..... DATE .....

.....

INPUT DATA

GRATICULE AREA .....mm^2 VOL cc

FILTER DIAM .....mm COUNT .....

COUNT

FIELDS	0	1	2	3	4	5	6	7	8	9
200										
190										
180										
170										
160										
150										
140										
130										
120										
110										
100										
90										
80										
70										
60										
50										
40										
30										
20										
10										
0										
COUNTS	0	1	2	3	4	5	6	7	8	9
COL TOTS										

OUTPUT DATA

FIBRE CONC FIBRES/mm^2 .....

C.V. .....

95%	
fpcc	
95%L	

REMARKS: .....

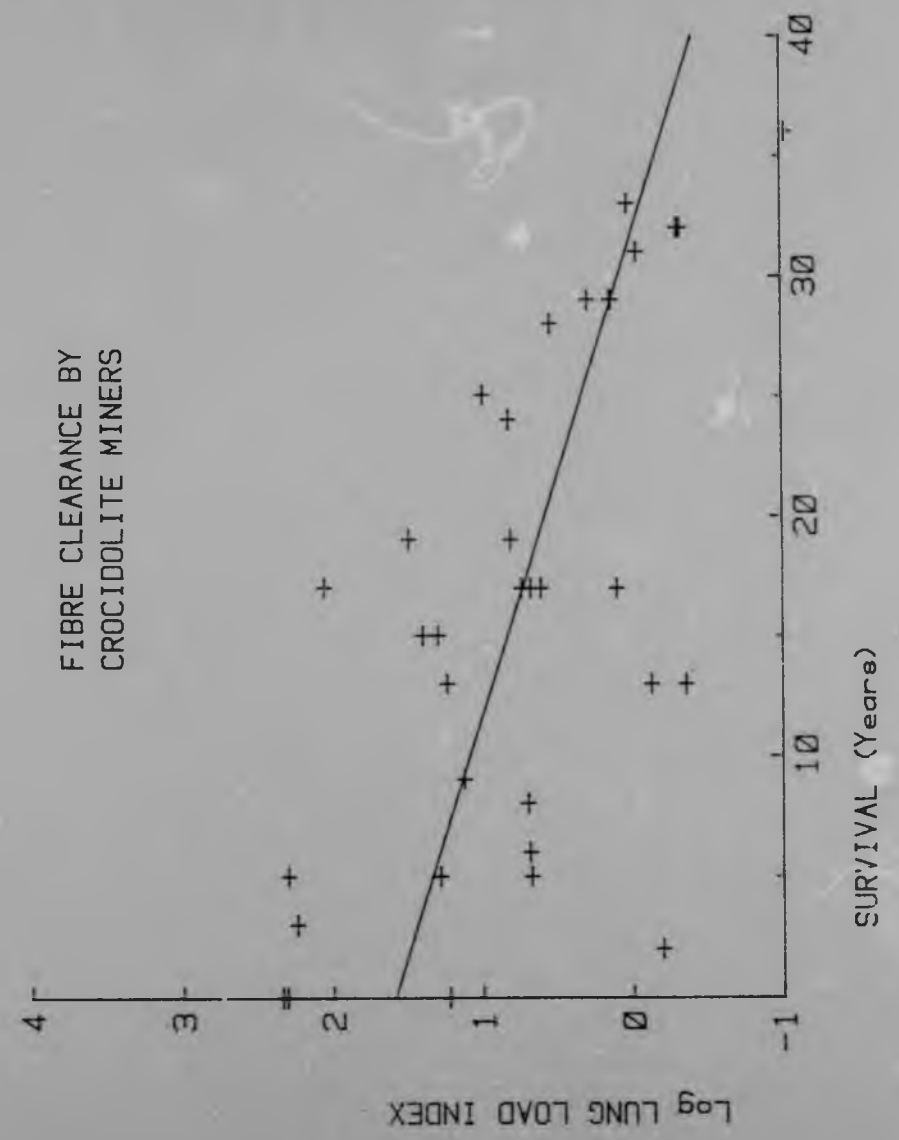
.....

.....

ELECTRON MICROSCOPE FIBRE COUNTING SHEET.

.....

APPENDIX D


FIBRE CLEARANCE BY
CROCIDOLITE MINERS
Log LUNG LOAD INDEX
4
3
2
1
0
-1
10
20
30
40
SURVIVAL (Years)