WORKING FOR A HEALTHY FUTURE

HISTORICAL RESEARCH REPORT

Research Report TM/91/05
1991

Further studies on the importance of quartz in the development of coalworkers' pneumoconiosis. Final report on CEC/BCC project 7248-33-044

Davis JMG, Addison J, Brown GM, Jones AD, McIntosh C, Miller BG, Whittington M

World Health Organisation
Collaborating Centre
For Occupational Health

HISTORICAL RESEARCH REPORT
Research Report TM/91/05
1991

Further studies on the importance of quartz in the development of coalworkers' pneumoconiosis. Final report on CEC/BCC project 7248-33-044

Davis JMG, Addison J, Brown GM, Jones AD, McIntosh C, Miller BG, Whittington M

INSTITUTE OF OCCUPATIONAL MEDICINE
Research Avenue North, Riccarton, Edinburgh, EH14 4AP
Tel: +44 (0)870 850 5131 Fax: +44 (0)870 850 5132
e-mail publications@iomhq.org.uk

FURTHER STUDIES ON THE IMPORTANCE OF QUARTZ IN THE DEVELOPMENT OF COALWORKERS' PNEUMOCONIOSIS

JMG Davis, J Addison, GM Brown, AD Jones
C McIntosh, BG Miller, M Whittington

August 1991
IOM Report TM/91/05

Report No. TM/91/05

INSTITUTE OF OCCUPATIONAL MEDICINE LTD

FURTHER STUDIES ON THE IMPORTANCE OF QUARTZ IN THE DEVELOPMENT OF COALWORKERS' PNEUMOCONIOSIS

by

JMG Davis, J Addison, GM Brown, AD Jones, C McIntosh,
BG Miller, M Whittington

FINAL REPORT ON CEC RESEARCH CONTRACT 7248-33-044

Price : £40.00 (UK)
£45.00 (Overseas)

August 1991

This report is one of a series of Technical Memoranda (TM) distributed by the Institute of Occupational Medicine Ltd. Current and earlier lists of these reports and of other Institute publications are available from the Librarian/Information Officer.

CONTENTS

Report No: TM/91/05

INSTITUTE OF OCCUPATIONAL MEDICINE LTD

FURTHER STUDIES ON THE IMPORTANCE OF QUARTZ IN THE DEVELOPMENT OF COALWORKERS' PNEUMOCONIOSIS.

by

JMG Davis, J Addison, GM Brown, AD Jones,
C McIntosh, BG Miller, M Whittington

SUMMARY

1. Coalworkers' pneumoconiosis is known to be related to the mass of coalmine dust inhaled but differences in dust composition create anomalies that are at present not fully understood. Thus, while quartz is known to be a very pathogenic material, the mass and proportion of quartz in inhaled dust does not always relate closely to the occurrence of pneumoconiotic nodules. The reasons for this are uncertain but there have been suggestions that free quartz surface, rather than total quartz, is related to disease development or that some dusts contain minerals that protect against the effects of quartz. The illite group of clay minerals has been particularly mentioned in this context.

2. The present study was undertaken using rats in order to examine the ability of coalmine dusts containing similar high quartz levels but different amounts of other minerals to produce pneumoconiosis. The rats were exposed by inhalation.

3. Large amounts of dust were collected from two British coalmines, one in Nottinghamshire where miners are known historically to have very low levels of pneumoconiosis, and one in Scotland which had mined a seam associated with a rapidly progressing pneumoconiosis of silicotic type. The dusts from the two mines contained similar proportions of quartz but very different levels of illite.

4. Batches of dust for use in the inhalation experiments were produced by mixing individually collected bulk samples from the Nottinghamshire and Scottish coalmines respectively. The respirable dust from the Nottinghamshire mixture contained 15.7% quartz, 18.6% kaolinite and 32% illite, while the respirable fraction from the Scottish dust contained 18.4% quartz, 25.5% kaolinite and 17.0% illite.

5. Airborne dust clouds were generated in whole body inhalation chambers at 20mg/m^3 respirable dust. The rats were exposed for seven hours a day, five days a week for periods of up to 18 months.

6. To examine the development of pulmonary pathology, rats were exposed to dust for either 12 or 18 months with follow up times of 16 and 10 months respectively. To study the development of early pulmonary inflammation, rats were exposed for periods of up to 30 days with a 30 day recovery period.

7. Following short periods of inhalation, dust from the colliery in Nottinghamshire associated with low levels of pneumoconiosis caused a greater inflammatory response than dust from the Scottish colliery associated with rapidly progressing pneumoconiosis. The Nottinghamshire dust caused a more marked and persistent influx of both macrophages and neutrophils into the lung and when extracted by bronchoalveolar lavage, cell populations from animals treated with the Nottinghamshire dust showed a greater reduction in chemotaxis *in vitro*. On a per cell basis, lavaged leukocytes from both dust treatments showed similar ability to degrade fibronectin. Since, however, the Nottinghamshire dust caused much greater cell recruitment, the overall protease burden in lungs treated with this dust was very much greater.

8. Treatment of rats for different periods of either 12 or 18 months did not result in significantly different levels of pathology when the lungs of these animals were examined at the end of the study (28 months). However, the pulmonary reaction caused by the dusts from the two collieries was different. In the rat lung, inhalation of the Scottish dust was followed by the formation of fibrotic nodules similar to those of pneumoconiosis or silicosis, while the Nottinghamshire dust failed to produce nodules. These results are entirely consistent with previous inhalation studies of a similar Scottish dust and with findings from human epidemiology, confirming that the differences observed in human populations from different collieries or coalfields are likely to result from differences in the dusts to which they are exposed rather than to other, e.g. geographical or environmental, differences.

9. Since the Scottish dust contained around 17% of illite, it must be concluded that the presence of this mineral type does not always prevent the development of silica related pneumoconiosis.

10. At the end of 12 months dust exposure there was evidence of increased retention of the mineral components of the dust, quartz, kaolinite and illite, and a corresponding reduction in the proportion of coal retained. Rats treated with the Nottinghamshire dust for either 12 or 18 months had a higher dust mass in their lungs at the end of dusting than those treated with the Scottish dust. This may be related partly to the fact that the overall particle size was finer with the Nottinghamshire dust since smaller particles are known to penetrate to the pulmonary parenchyma more easily than coarse ones. Incomplete digestion of tissue, particularly fatty residues, from rat lungs with advanced pathological change, limited the information that could be obtained from lung burden analysis.

11. The study has confirmed previous indications that different coalmine dusts with equal quartz levels may cause different tissue reactions. In this study, one dust caused a marked inflammatory response from the start of dust inhalation and eventually produced widespread diffuse fibrosis. The other dust showed less short term toxicity and although producing widespread diffuse fibrosis, also resulted in the formation of large numbers of small silicotic nodules. This finding may explain the results of several previous publications which failed to demonstrate a relationship between simple dust toxicity and pneumoconiosis. These two biological responses may result from substantially different processes. The exact mechanisms of nodule formation still remain to be elucidated but may be related in part to the mobility, particularly chemotactic mobility, of macrophages containing dust particles. In rats exposed to the Scottish dust macrophages accumulated around the perivascular lymphatics at the centre of the pulmonary acini – the site where nodule formation arises. In those rats exposed to the Nottinghamshire dust, the macrophages remained distributed throughout the alveolar region and did

not form the macrophage aggregations seen with the Scottish dust. The lack of nodule formation with the Nottinghamshire dust may therefore be related to an impaired ability to migrate to the perivascular lymphatics.

12. Although the respirable quartz mass concentrations in the inhalation chambers were similar and although the Nottinghamshire dust was finer and thus of greater surface area, the measured free quartz area of the Scottish dust was very much greater, giving strong support to the view that the pathogenicity of quartz, as it relates to pneumoconiosis, increases with its free quartz surface area. These results have implications for the setting of occupational exposure limits, since the risk of developing silicotic pneumoconiosis appears to be a function not only of the amount of quartz present, but also of its type or nature.

13. The association between the formation of fibrotic nodules in the rat lung in this experiment and radiological change in coalminers exposed to similar coalmine dust suggests that there may be a useful role for rat inhalation studies to study the ability of other dusts to produce pneumoconiotic changes. Further work should include comparisons with dusts such as anthracites, which are known to carry a high risk of pneumoconiosis although they typically contain little quartz. If the responses over a range of dusts are consistent with results from human epidemiology, it is conceivable that rat inhalation studies could be used in future to assess risks from dusts created in new or changed mining conditions.

1. INTRODUCTION

The role of the quartz content of coalmine dust in the development of coalworkers' pneumoconiosis has been the subject of much controversy and much research. The recognition of silicosis as a disease of workers exposed to dusts containing high levels of quartz predated the recognition of coalworkers' pneumoconiosis as a separate disease entity, and it is now well documented that quartz dust, as encountered by masons, stoneworkers etc., is highly pathogenic to the tissue of the human lung (Seaton et al. 1987). Indeed, when symptoms similar to those of silicosis were first documented in coalworkers, it was presumed that these were due to quartz in the coalmining environment, until studies in Wales in the 1940s described the disease in workers whose exposure was to virtually pure coal dust.

Extensive epidemiological studies of coalworkers in Britain and West Germany have shown a strong dose-response relationship between an individual's risk of developing coalworkers' pneumoconiosis and estimates of his cumulative lifetime exposure to respirable dust. Systematic differences have however, been observed in the prevalences of pneumoconiosis between collieries with similar levels of respirable dust, suggesting that some dusts are more likely to cause pneumoconiosis than others. The composition of coalmine dusts varies greatly between different mining areas. Where coal of high rank (particularly anthracite) is mined the coal itself may make up to as much as 95% of the respirable dust inhaled by miners. Where coal of low rank is mined, however, coal may be as low as 40% of the total with the rest of the respirable dust generated comprising various mineralogical elements. Quartz frequently makes up a large part of the mineral fraction of coalmine dusts.

In studies which have sought to compare results from different collieries, however, the severity and prevalence of coalworkers' pneumoconiosis over the full range of mining conditions has not appeared to relate directly to the quartz content of the respirable mine dust, and no single factor has emerged which could explain all the differences in prevalences between collieries. Highest risks have been observed in collieries mining high rank coal, that is coal with relatively low mineral content and many of the collieries in which the dusts contained relatively high proportions of quartz and other minerals exhibited lower risks of pneumoconiosis (Jacobsen *et al.* 1971; Reisner, 1971; Reisner and Robock, 1977; Walton *et al.*. 1977; Hurley *et al.* 1982). Other studies have demonstrated exceptions to this apparent trend, and some miners exposed to dust with high quartz content have shown unusually rapid radiological changes. This was demonstrated by Jacobsen and Maclaren (1982) and Hurley *et al.* (1982), using case-control procedures on 41 pairs of men. In addition, Hurley *et al.* (1982) showed that this quartz-related rapid progression was associated with a relatively frequent occurrence of PMF. In a separate study Seaton *et al.* (1981) observed rapid progression of pneumoconiosis in a group of miners at one Scottish colliery who were exposed to unusually high proportions of quartz in dust in a colliery where previously both quartz levels and the prevalence of disease had been lower than average. Miller and Kinnear (1988) reported on a detailed re-examination of the radiographs from this colliery, and confirmed that the rapid progression was related particularly to levels of exposure to quartz in the early 1970s.

In general, experimental studies - as reviewed in the next chapter - have confirmed that quartz plays a prominent part in determining the pathogenic potential of coalmine dust, but have also emphasised that many factors are

involved. *In vitro* studies have demonstrated that increasing toxicity is associated with increasing ash and quartz content of the dust although there have been important differences between results from studies using different techniques, and considerable scatter has been observed in relationships between mineral content and dust pathogenicity. More recent work has centred on investigating the short-term inflammatory response to coalmine dust in the lung. This approach has demonstrated that exposure to coalmine dust results in the accumulation of inflammatory leukocytes in the bronchoalveolar region. These cells have the ability to cause tissue injury (Brown and Donaldson, 1989) and can contribute to the development of lung fibrosis (Hunninghake *et al.* 1984). Although pure quartz is known to be markedly more inflammogenic than coalmine dust (Donaldson *et al.* 1990[a]), no evaluation has yet been made of the contribution of the quartz component of coalmine dust in eliciting an inflammatory response in the lung.

At least part of the explanation for these variable findings may be due to the presence of other minerals, including coal and types of clay, which have been reported to inhibit the toxic action of quartz. It has been suggested that this is due to the absorption of 'protective' substances onto the reactive surfaces of quartz particles and Reisner (1983) has shown that there is a better correlation between coalmine dust toxicity and uncontaminated quartz surface area (measured by thermoluminescence) than between toxicity and the quartz mass content of the dusts. Le Bouffant *et al.* (1983) have also shown experimentally that such inhibition may not be permanent and suggested that continued exposure may maintain protection whereas removing a man from exposure to coalmine dust may result in the termination of the inhibition of quartz toxicity by other minerals.

The importance of the quartz content of coalmine dust in a situation where the amount of other, possibly protective, minerals was relatively constant has been demonstrated by Robertson *et al.* (1984). In that study, samples of coalmine dust were collected from a colliery mining the same seam as that reported on by Seaton *et al.* in 1981. The daily quartz content of the mine dust was known to vary considerably and by collecting individual daily dust samples, it was possible to accumulate a high quartz sample with a quartz content of almost 25% and a low quartz sample containing only 6%. These dusts and an intermediate mixture of the two with a quartz content of about 12% were administered to groups of rats by inhalation at a dose level of 20mg/m^3 of air. The study confirmed the importance of the quartz content of the dust, since by the end of the 12-month inhalation period the high quartz sample had produced multiple pulmonary pneumoconiotic nodules while the low quartz dust had produced almost none. Subsequently some pneumoconiotic nodules developed in animals treated with the low quartz dust but the numbers in the high quartz group also increased with time and a differential was maintained. The numbers of nodules produced by the medium quartz dust was in between the high and low quartz figures.

The study of Robertson *et al.* (1984) thus demonstrated that the rat lung can be made to respond to exposure to airborne dust by producing nodules similar to those characterising coalworkers' pneumoconiosis and, moreover, showed that it was possible to obtain in this situation an apparent relationship between the ability of the dusts to produce nodules and the quartz content of the dusts. Since the dusts used were collected from the same seam as had been implicated in the rapid progression of pneumoconiosis in an adjacent Scottish colliery, these findings were very consistent with the epidemiological results of Seaton *et al* (1981) and Miller and Kinnear (1989).

It remained uncertain, therefore, why dusts in collieries in areas such as

Nottinghamshire, which often contained quartz in proportions up to 20%, should have been associated with relatively low prevalences of pneumoconiosis. To obtain further information, it was decided to collect bulk samples of dust from such a Nottinghamshire colliery and to undertake long-term inhalation studies in rats similar to those performed by Robertson *et al.* in 1984. In addition, short periods of inhalation would be used to explore the effects of this dust on the behaviour of the leukocyte populations in the bronchoalveolar region of the lung. For comparison, similar investigations would be carried out using fresh collections of dust from the Scottish colliery, chosen to have similar quartz content to that of the dust from Nottinghamshire. The Scottish colliery dust had much lower content of the illite clay minerals, while high levels were known to exist in many Nottinghamshire collieries, permitting an examination of possible protective effects.

2. SUMMARY OF OBJECTIVES

The study reported here had the following objectives:

1. To expose the lungs of rats to two coalmine dusts of similar quartz content, one dust from a mining area with low risks of pneumoconiosis, and the other from an area where rapid progression of pneumoconiosis had been observed in workers.

2. To examine whether the pathological responses of the rat lungs to the two dusts, after short-term and longer-term exposure, were consistent with the epidemiological findings in the areas from which the dusts originated.

3. To relate any differences in these responses to the mineralogical composition of the dusts, paying particular attention to the role of illite.

3. LITERATURE REVIEW

Coalworkers' pneumoconiosis is a fibrotic condition caused by the inhalation by coalminers of dust generated during the mining of coal. The earliest lesions result from the simple accumulation of dust in the lungs. As demonstrated by Heppleston in a series of papers (1947,1951,1953,1954) the early dust lesions developed from the accumulation of dust-laden macrophages around the divisions of the respiratory bronchioles. With the passage of time reticulin fibres are laid down amongst the cellular aggregates, binding the cells together and in more advanced lesions collagen fibres are often present. With the ageing of the connective tissue shrinkage occurs. At this point there can be 2-5 dust "macules", 1-4mm in diameter, in each secondary lobule. The individual dust deposits, however, remain small so that in fresh lung specimens the macules feel soft to the touch. In more advanced cases a continued deposition of dust in association with connective tissue fibres can lead to solid masses, 2-10mm in diameter, which are hard to the touch and give the lung a lumpy feel. Nodules reaching a size of more than 10mm in diameter are classed as progressive massive fibrosis.

The composition of coalmine dusts varies greatly between different mining areas and the specific role of dust composition in the development of this disease is as yet not fully elucidated although it has been the subject of considerable research.

In 1945 King and Nagelschmidt published a report of the examination of lungs from 54 coalminers in South Wales. The degree of pulmonary pathology was compared to the lung dust content and the dust analytical procedures included estimations of coal, quartz, kaolin and mica. They reported that while the lung dust of some men contained over 95% coal, in others who were classified as rock workers, the coal level was as low as 25% with a corresponding increase in other minerals. It was found that for a group of 27 anthracite miners, the composition of the lung dust was very similar to that of dust from the coalface and the authors therefore concluded that there was no mineralogical change in the lung. It was found that the concentrations of coal and quartz increased only very slightly from one pathological group to the next with the exception of those rock workers with silicotic nodules. Later in 1956 King, Maguire and Nagelschmidt reported similar studies on a further series of 71 coalminers' lungs from men who had worked in pits covering all the ranges in coal rank found in South Wales. In addition, 15 cases of silicosis from Cornish tin mines were included. Pathological and mineralogical analyses were undertaken as before but on this occasion, for 30 of the cases, the chest radiograph was compared to the level of pathology found at autopsy. The results for the group of coalminers was similar to the earlier study. While the total lung dust increased with increasing pathology, the quartz percentage did not change. The authors therefore concluded that quartz was not a significant factor in the development of progressive massive fibrosis and they favoured the idea that a secondary factor, usually tuberculosis, was required in addition to dust in order to produce this condition. It was pointed out, however, that the lungs of coalminers usually contained more quartz than tin miners with pronounced silicosis and it was suggested that the coal dust diluted the quartz and therefore reduced its effect.

These studies were later enlarged to include the examination of lungs from miners in the Cumberland coalfield (Faulds, King and Nagelschmidt, 1959) and the Lancashire coalfield (Spink and Nagelschmidt, 1963). In both areas it was found that the lung dust from all grades of pneumoconiosis had a higher percentage of

quartz than had been found in South Wales. In these areas there was some evidence of a progressive rise in the % quartz with increasing levels of pathology although in Cumberland no cases were reported that the authors recognised as classical coalworkers' progressive massive fibrosis. In both areas, however, some men often classified as rock workers had a very high percentage of quartz in their lung dust and lesions similar to silicosis. In some cases the silicotic nodules had fused to produce what was termed "silicotic massive fibrosis". In 1963 Nagelschmidt *et al.* specifically examined lungs from cases of progressive massive fibrosis for evidence of the importance of quartz in the development of these lesions. They reported that while the total dust content of PMF lesions was on average twice as high as the rest of the lung tissue, the percentage quartz in the dust from the two samples was almost identical. The mean quartz content of 32 cases of PMF was slightly higher than that of 58 cases of simple pneumoconiosis but the difference was not significant. It was concluded that quartz had not been an important factor in the development of progressive massive fibrosis in the cases examined. A similar increase in the total content of quartz in PMF lesions was observed by Vyskocil *et al.* in 1970.

Studies relating the levels of pneumoconiosis in coalminers to the varying dust parameters have now appeared from several countries. In the USA, Naeye *et al.* (1971) and Naeye and Dellinger, (1972) reported the results of studies in which pneumoconiotic lesions from the lungs of miners working with different ranks of coal were compared with the levels of "silica crystals" in the lungs. These were estimated by a counting procedure using polarised light and the authors admitted that their counts represented the lung content of total silicates and not quartz. They reported that the highest level of "silica crystals" were present in the lungs of men who had mined high rank coal and that these men had the highest levels of pneumoconiosis. In 1974, Sweet *et al.* published the results of a study in which they had examined the lungs of a group of bituminous coalminers from the USA. Pathology was compared to detailed mineralogical analysis of the levels of coal, non-coal minerals and quartz, but unfortunately only sections of the lungs were analysed and the mineral levels had to be expressed as g/100g of dried lung tissue. It was found that the levels of both coal dust and free silica increased with increasing levels of pathology.

Davis *et al.* (1979) and Ruckley *et al.* (1981) in an autopsy study of the lungs of over 500 coalminers from all the mining areas of Britain demonstrated that while total dust mass related to the severity of pneumoconiotic lesions, this was much more marked in miners from collieries mining high rank coal. As reported by previous workers, it was found that the composition of retained lung dust from men who had mined high rank coal varied little between the earliest lesions and advanced progressive massive fibrosis. In men who had worked with low rank coal, however, there was a progressive increase in ash and particularly quartz content between the earliest recognisable dust lesions, hard fibrotic nodules and progressive massive fibrosis. At least part of this increase of quartz with increased pathology appeared due to a selective retention of quartz particles in the lung. Compared to the dust to which men had been exposed, cases of PMF had on average more than double the percentage of quartz in their lung dust at autopsy.

As with the direct comparison of dust content and related lung pathology in lung tissue, epidemiological studies have demonstrated a relationship between mine dust composition and the prevalence of pneumoconiosis but have been unable to elucidate all the factors involved. Studies in Britain and West Germany have given similar results (Jacobsen *et al.* 1971; Reisner, 1971; Reisner and Roebock,

1977). The prevalence of coalworkers' pneumoconiosis is strongly associated with cumulative exposure to respirable dust but there are large differences in response to dust exposure between collieries. Some effects of the coal rank and mineral composition of the dust, particularly the quartz content, were noted but the correlation between prevalence and exposure is not generally improved by making allowance for these factors (Walton *et al.* 1977; Hurley *et al.* 1982). However, it has recently been demonstrated that miners exposed to dust with high quartz content may show unusually rapid radiological changes. This has been demonstrated by Jacobsen and Maclaren (1982) and Hurley *et al.* (1982). In addition, Jacobsen and Maclaren (1982) showed that this quartz-related rapid progression was associated with a relatively frequent occurrence of progressive massive fibrosis (PMF). In separate studies Seaton *et al.* (1981), Miller *et al.* (1988) observed rapid progression of pneumoconiosis in a group of miners at one colliery who were exposed to unusually high proportions of quartz in dust in a colliery where previously both quartz levels and the prevalence of disease were low.

One of the problems of epidemiological studies of coalworkers' pneumoconiosis is that diagnosis of this condition and grading of its severity during life relies largely on chest radiographs and there is some doubt as to whether all apparently similar opacities seen in coalminers really represent exactly comparable pathological lesions. As far back as 1942, D'arcy Hart and his co-workers demonstrated that the early stages of coalworkers' pneumoconiosis produced a pattern of fine reticulation on a radiograph and since that time the presence of this reticulation has been recognised as indicating a level of pneumoconiosis for which compensation may be awarded. However, doubt remained for some time as to exactly which aspects of the disease resulted in the reticulation. Gough, James and Wentworth (1949) summarised the situation at that date and suggested that both the "granules" and "pinhead opacities" reported by other workers were due to the presence of coaldust nodules. They concluded that the degree of focal emphysema associated with the dust nodules could not be accurately assessed by radiography but that the sharply defined net-like appearance in radiographs corresponded with severe focal emphysema. In 1945 Sutherland suggested that the radio-opacity of coaldust nodules alone would be insufficient to cause a pattern of reticulation from radiographs without the contrasting effect of accompanying focal emphysema. However, Gough, James and Wentworth were able to show that nodules could be demonstrated by X-ray in the complete absence of emphysema and concluded that the fibrous tissue of the nodules contributed more to their radio-opacity than the mineral content. Kaplan in 1962 showed that for a group of coalminers from South Wales, the size and number of dust nodules correlated well with radiographic category. He found that no cases of category 0 and only 11% of category 1 had fibrotic nodules. The figures for categories 2 and 3 were 44% and 77% respectively. It was reported that no case had large numbers of fibrotic nodules but no dust analyses were undertaken so that the effects of mineralogical content on radiographic categories could not be examined.

This problem was further considered in a series of studies by Rivers *et al.* (1960); Rossiter *et al.* (1967); Casswell *et al.* (1971) and Rossiter (1972). Initially, Rivers *et al.* concluded that in miners from South Wales the mineral content of inhaled dust contributed weight for weight about nine times more to X-ray opacity than coal. In later papers, however, the authors reported on a larger series of lungs from a wider range of coalfields and concluded the differential was only 3:1. It was admitted, however, that much of this anomaly was due to the exclusion of category 0 and 3 from the earlier analyses. In these later studies, good correlation between dust and radiographic category was found in most cases but two

small sub-groups gave anomalous results. The first group consisted of lungs from Scottish coalminers who had a higher radiographic classification than their dust content appeared to warrant but the authors concluded that this was due to the inhalation of soot from naked flame lamps. The second sub-group consisted of cases with "nodular" sized opacities which were again given a higher radiographic category than would have been expected from their dust content.

In Germany, both Einbrodt (1965) and Worth *et al.* (1968) showed that the radiographic category of coalworkers' pneumoconiosis (silicosis) tended to increase with increasing total dust. Worth and his colleagues concluded however, that the pneumoconiotic shadows were not caused directly by the inhaled dust but by the fibrous tissue reaction. Naeye and Dellinger (1972) reported on the lungs of 77 bituminous coalminers and found that the correlation between radiographic categories and the volume of pulmonary dust nodules was good. They also indicated that the lung content of silicon dioxide correlated well with the radiographic category but, unfortunately, this material was estimated by a light microscope counting process and not chemical analysis. In 1970, Ryder *et al.* published the results of a large study in which the lungs of 247 coalminers had been examined and radiographic category was compared not only to the levels of pneumoconiosis present, but also to the levels of emphysema. It was found that higher levels of emphysema were present in those pneumoconiosis cases showing the finer punctiform type of radiological change than in cases showing the larger micro-nodular and nodular opacities.

Much experimental work has been undertaken in the attempt to understand the relationship between mineralogy and the development of coalworkers' pneumoconiosis.

Early *in vivo* studies by Belt and King (1945) reported little reaction following intratracheal injection of coaldusts but stated that any reaction occurring was greatest with the high mineral content dusts. In 1956 Attygale *et al.* showed that quartz plus coaldust was more fibrogenic than coal alone. More recent studies (Schlipkoter *et al.* 1971; Le Bouffant *et al.* 1977) have demonstrated increasing pathological effects with increasing quartz contents of dust using artificial mixtures. The presence of other minerals including coal and clay minerals has been reported to inhibit the toxic activity of quartz (Le Bouffant *et al.* 1977,1983). Kriegseis *et al.* (1977) suggested that this is due to absorbed species on the quartz. Subsequently it was demonstrated that correlation was better between coalmine dust toxicity and uncontaminated quartz surface area (measured by thermoluminescence) than between toxicity and the quartz content of the dusts (Reisner, 1983). Le Bouffant *et al.* (1983) have also shown experimentally that such inhibition may not be permanent and suggested that continued exposure may maintain protection whereas removing a man from exposure to coalmine dust may result in the termination of the inhibition of quartz toxicity by other minerals.

Because of the great expense of long-term animal studies, much effort has been spent in attempting to use *in vitro* tests to examine mine dust toxicity or to devise short-term *in vitro* tests that could predict the risk of pneumoconiosis occurring from any particular colliery. *In vitro* studies have demonstrated large differences in the short-term toxicity of mine dusts. Generally, increasing toxicity is associated with increasing ash and quartz content of the dust (Reisner and Robock, 1977; Gormley *et al.* 1979; Addison *et al.* 1982). Gormley *et al.* (1979) also suggested that this relationship was closer with high rank coalmine dusts and for given quartz content the toxicity of a high rank dust would appear to be much higher. Similar results have been obtained from a variety of *in vitro* tests (Bruch

et al. 1975,1983; Reisner, 1983; Weller and Rosmanith, 1983). Many of these workers and others combined in a project to examine the same series of mine dusts by their own preferred tests. The results were reported by Le Bouffant *et al.* (1988) but did not indicate very close correlation between information from the different procedures.

More recent experimental work has concentrated on assessing the short-term inflammatory response in the lungs of animals exposed to coalmine dust. The rationale behind this approach was the advent of the technique of bronchoalveolar lavage (BAL) as a tool in assessing lung inflammation in human subjects. It was found that this technique could be reliably used in the diagnosis and staging of interstitial lung disease (Crystal *et al.* 1981) and it has subsequently been applied in the assessment of coalworkers' pneumoconiosis where inflammatory leukocytes were demonstrated in the BAL fluid (Sablonniere *et al.* 1983; Voisin *et al.* 1985). Experimental studies have also demonstrated the accumulation of inflammatory leukocytes, macrophages and neutrophils, in the bronchoalveolar lavage fluid of rats exposed to coalmine dust by inhalation (Brown and Donaldson, 1989) or by intratracheal instillation (Bingham *et al.* 1977). In the inhalation model, the inflammatory response is associated with the pathogenic potential of the dust; those dusts which are most fibrogenic also cause the greatest accumulation of inflammatory bronchoalveolar leukocytes in the short-term (Donaldson *et al.* 1988).

The relationship between the inflammogenic potential of a dust and its ability to cause fibrosis rests on the central role of inflammatory leukocytes in tissue injury and control of cell proliferation. The toxic products of leukocytes, proteolytic enzymes and reactive oxygen species, can damage the surrounding lung tissue, while inflammatory mediators induce cell proliferation and secretion of connective tissue molecules. In the short-term, inflammatory leukocyte-mediated tissue damage resolves, resulting in the restoration of normal tissue architecture. In conditions of chronic lung inflammation, however, this delicate balance is upset, leading to altered deposition of connective tissue components during repair processes and so ultimately to lung fibrosis (Hunninghake *et al.* 1984).

An important function of the resident bronchoalveolar leukocytes is clearance of particles deposited in the lung to the mucociliary escalator and to the lymphatic system. This function is important in limiting the harmfulness of the particles by removing them from the delicate alveolar surfaces. In order to remove particles from the alveolar region, the leukocytes must be able to move. This function can be tested *in vitro* by assessing the ability of the leukocytes to migrate in response to a chemotactic stimulus (Donaldson *et al.* 1989). There is no information available at present on the chemotactic activity of BAL leukocytes from pneumoconiotic subjects. However, impaired chemotaxis has been demonstrated in bronchoalveolar leukocytes from rats exposed by inhalation to the pneumoconiotic dusts quartz, coalmine dust and asbestos but not in those exposed to the non-fibrogenic dust titanium dioxide (Donaldson *et al.* 1990[b]).

In coalworkers' pneumoconiosis, the BAL fluid has been found to contain increased levels of proteolytic enzymes (Sablonniere *et al.* 1983), reactive oxygen species (Voisin *et al.* 1985), inflammatory mediators (Borm *et al.* 1988) and fibroblast growth factors (Rom *et al.* 1987). Although there has been little experimental work on the functional activity of BAL leukocytes following exposure to coalmine dust, there are differences in rat BAL leukocytes in secretion of inflammogenic agents (Kuhn *et al.* 1990). In addition, it has been shown previously that these cells have increased proteolytic enzyme activity (Brown and Donaldson, 1989).

Thus differences in cell numbers and in the functional activity of the leukocytes which accumulate in the bronchoalveolar region in response to a given dust exposure may provide an indication of the pathogenic potential of that dust. In the present project, the short-term inflammatory response to the two dusts under study has been evaluated by assessing total number and types of leukocytes in the BAL fluid. Two aspects of leukocyte function have also been evaluated, one beneficial and one potentially harmful, that were deemed particularly relevant to the pathogenic potential of the dusts. Firstly, the ability of the leukocytes to chemotact and so remove dust particles from the lung, thus reducing the duration of the biological reactivity of the dust was measured. Secondly, the *in vitro* proteolytic activity of the cells was measured which is an indicator of their ability to inflict tissue damage as noted above.

In 1984 Robertson *et al.* undertook a long-term inhalation study to examine experimentally the effect of dusts with high quartz levels reported to produce rapid progression of pneumoconiosis in Scottish coalminers (Seaton *et al.* 1981). Rapid development of pneumoconiosis had been detected in a group of miners who had suffered unusually high quartz exposure and it was felt that by examining dusts of varying quartz content from the same mining location the exact relationship of pathology to quartz content could be determined at least in regard to this one mining situation. The colliery reported on by Seaton *et al.* had closed before these dusts were required but they were obtained from another colliery three miles away which mined the same coal seam. By collecting and analysing separate dust samples daily, three bulk batches of dust were mixed with quartz levels of 6.8%, 12.9% and 24.5% respectively. Rats were exposed to these dusts for up to 18 months and discrete-pigmented nodules developed in their lungs. The highest nodule profusion was found in those animals exposed to the high quartz dust and these animals also produced nodules earlier than the other groups. Exposure to the low quartz dust produced very few of these lesions at any time of the animal's life. With the dust from this particular mining situation, therefore, levels of pneumoconiosis found in both humans and experimental animals appeared directly related to quartz content. One factor with these mine dusts, however, was their low content of illite, levels of which were only 2-3% of the total. Le Bouffant *et al.* (1977) have reported that these clay minerals can exert a pronounced protective effect against the development of quartz-related lesions but his studies were undertaken with artificial dust mixtures.

4. MATERIALS AND METHODS

4.1 Colliery Selection

This project required relatively large amounts of respirable dust from two coal mines, both with a high quartz content, but with significantly different illite contents.

The Institute of Occupational Medicine had already undertaken long-term inhalation studies with rats using dusts from a Scottish colliery (Robertson *et al.* 1984). This colliery was adjacent to one previously reported to have a high progression rate for pneumoconiosis associated with high quartz exposures (Seaton *et al.* 1981). The same seam was mined in both collieries and while quartz levels in respirable mine dust varied, some could be well over 20%. Among the range of dusts tested from this colliery the "high quartz" specimen contained 24.5% of quartz and a very low proportion of illite. The results of the experimental study demonstrated that this dust could cause the development of pneumoconiotic nodules in rat lungs and this information coupled with the human data led to the colliery being selected to provide one of the dusts for the present study.

The Nottinghamshire area of the British mining industry was chosen to provide a second colliery needed in the present study. This area generally had a high proportion of quartz in mine dusts but also high illite contents. The prevalences of pneumoconiosis in the area have been generally among the lowest in the country. The colliery finally selected had worked the same seam for 50 years so that there was a good chance that the respirable dust samples collected for use in the present study would be of similar composition to the dust to which miners in the past were exposed. Quartz contents in respirable dust were known to reach more than 20% on some occasions and even higher illite contents of the same order could also be expected.

4.2 Dust Collection and Analysis

In both collieries large amounts of dust were collected in return roadways using an auxilliary ventilation fan to drive air through dry fabric filters. Once the filters had become laden with dust they were sent to the IOM in Edinburgh where the dust was removed from the fabric using a vacuum cleaner. During roughly the same period of time, separate dust samples from both collieries were collected as part of a larger project on the size distributions of the various components of mine dusts. Full details of this work have been reported by Addison *et al* (1990).

Collection of an excess of dust over that needed for the inhalation study permitted selection and mixing of dust from different filters to obtain the most desirable combination. To provide the necessary information on the quartz content of the dusts, respirable dust clouds were generated from a small amount of each filter sample and collected for analysis using the Casella MRE113A dust sampler which samples in accordance with the BMRC Johannesburg definition of respirable dust (Orenstein, 1960). The dust was subsequently scraped from the membrane filter used in this apparatus and incinerated in a muffle furnace at 380°C to determine its ash content by weight loss. The residue was then ground to produce a uniform mixture with approximately 250mg of potassium bromide and compressed into the form of a disc for mineralogical analysis by infrared spectrometry (Dodgson and Whittaker, 1973).

Following analysis of the individual dust filter batches, selected batches were mixed by tumbling in a large plastic container to produce a large quantity of material that it was hoped would permit generation of dust clouds of uniform quartz composition throughout the experimental inhalation periods.

To prevent oxidation, dusts for use in the generation of respirable dust clouds were stored in plastic containers under nitrogen until needed. Since prolonged storage of some of the dust containers was envisaged, each batch of dust was pasteurised by a 1500 Gy radiation dose of gamma radiation from a Cobalt 60 source (Robertson *et al.* 1984).

During the experimental phase of this project, dust was collected using the MRE 113A sampler each day to determine the respirable mass concentrations of dust. Total dust concentrations were measured on 20 days only throughout the project to determine the relative proportion of coarse dust in the airborne clouds. Total dust samples were collected using a downward facing 50mm diameter filter holder at a flow rate of 2 l/min.

The quartz composition of the respirable dust clouds generated was assessed by analysing a representative section of the daily samples collected by the MRE 113A. In practice a total of 30 analyses of dust from the Scottish colliery were undertaken during the inhalation period of the study and 27 analyses of dust from the Nottinghamshire colliery. These samples were each analysed by infrared absorption techniques following ashing at 380°C (Dodgson and Whittaker, 1973). Three samples from each colliery, collected as part of the daily gravimetric evaluation of the dust clouds were subjected to infrared analysis following ashing at 700°C as well as at 380°C to enable the illite, kaolinite and quartz levels to be determined (Addison *et al.* 1982).

4.3 Free Quartz Surface Area

Free quartz surface areas of respirable fractions of the dusts (MRE type 113A) were measured using thermoluminescence and radioluminescence techniques by Dr W Kriegseis of the University of Giessen, Germany (Kriegseis and Scharmann, 1982). Only relative "free quartz surfaces" for the two dusts could be derived because actual quartz or other mineral contents were not available at the time of analysis. However, since the quartz and other mineral contents were in fact similar it is suggested that the relationships between these areas are close to those between actual free quartz areas. The thermoluminescence (TL) measured was light emitted by the dust samples during heating from 77°K having been previously irradiated by x-rays at that temperature. The radioluminescence (RL) was light emitted and measured during heating-up of the dust samples and during x-ray irradiation at a constant dose rate. Free quartz surface area as measured by TL and RL was suggested by Kriegseis and Scharmann (1982) to be directly related to quartz toxicity as measured by cytotoxicity studies. Further details of the techniques are given in the Appendix.

4.4 Experimental Protocol

4.4.1 Main inhalation study

Two groups, each of 48 male specific pathogen free rats of the Wistar Han strain were used for the inhalation study aged approximately 12 weeks at the start of the experimental period. All were born within a one week period and were randomly allocated to each group. These rats were caged in groups of 12 during the

inhalation period and in pairs thereafter and given *ad libitum* access to a standard pelleted laboratory diet (SDS Limited) and tap water throughout the period of study.

Respirable dust clouds of the 2 mine dusts were generated using a Wright dust feed (Wright, 1950) in inhalation chambers previously described by Beckett (1975). The Nottinghamshire dust contained less coarse dust that that from the Scottish colliery and therefore required a smaller volumetric feed rate of powder to achieve the target respirable dust concentration. The Wright dust feed used for this dust was therefore set up with a smaller bore tube to provide the required feed rate.

The planned exposure levels for both colliery dust samples was 20mg/m^3 respirable dust and both groups of rats were initially exposed for 12 months with dust generated for 7 hours each day and 5 days each week. At this point, 18 animals from each group were removed from the dust chambers. Six were killed and the remaining 12 maintained in normal laboratory cages in a dust-free environment. Dusting was terminated after 18 months for all animals at which point 6 animals from each exposure group were killed on removal from the dusting chambers and 4 from each of the two batches removed from dust after 12 months. The remaining animals were maintained for most of the rest of their natural lifespan although those showing signs of debilitation were killed at any time. This experimental regimen is illustrated in Figure 1. The experiment terminated 28 months from the commencement of dust exposure. At this point survivors were 31 months old.

4.4.2 Short-term inhalation to examine early pulmonary response

The removal of 18 rats from the inhalation chambers, after 12 months of dusting in the long-term study, liberated one cage in each chamber. This space was utilised by exposing three month old male rats of the same AF Han strain to the two coalmine dust clouds for short periods of time to examine the development of pulmonary inflammation. Animals were given 3,7,15 or 30 days of actual dust exposure. Thus fifteen working days covered an elapsed period of 3 weeks (five working days each week plus two weekend days). After dust exposure groups of 4 dust-exposed and 4 control rats were killed and the inflammatory bronchoalveolar response assessed. In addition, groups of 4 rats were removed from the chambers at each time point and maintained in room air for a period of 30 days to assess the effect of a "recovery" period on the inflammatory response. This experimental regimen is shown in Figure 2. In this case, however, the 30 days were elapsed time, i.e. four weeks and two days. Bronchoalveolar leukocytes were retrieved by lavage as previously described (Brown and Donaldson, 1988). Briefly, the lungs were resected and lavaged with 4 aliquots of saline at 37 ^{0}C. The lung washes were pooled and placed immediately on ice prior to centrifugation and washing with phosphate buffered saline. The following studies were undertaken with the lavaged cells:

1. Cell counts
Total cell and differential counts were undertaken using trypan blue and May-Grunwald-Giemsa stained preparations respectively. Total cell counts were undertaken using a haemocytometer chamber.

2. Bronchoalveolar leukocyte function
The functional status of the leukocytes was assessed at each time point by measuring, *in vitro*, various parameters of the cells which can be modulated during inflammation. Proteolytic activity was measured by assessing ability to

degrade fibronectin (Brown and Donaldson, 1988). Cells were diluted to a concentration of 5 x 10^5/ml in Ham's F10 medium (Life Technologies, Paisley) containing 0.2% bovine serum albumin (Sigma, Poole, Dorset) and were then cultured as 0.2ml of cell suspension/well (3 wells per animal) in microtitre plates coated with iodinated fibronectin. The plates were then incubated for 4h at 37 ^{0}C. Fibronectin breakdown was measured as release of radioactivity into the culture medium.

The ability of the leukocytes to respond to a chemotactic gradient was measured using Boyden chemotaxis chambers (Donaldson *et al.* 1990). Two hundred microlitres of 5% zymosan-activated rat serum were placed in the lower compartment of the chemotaxis chamber and a membrane filter, of 5μm pore size (Nuclepore, Pleasanton, CA), was placed on top. Four hundred microlitres of RPMI medium containing 1% Bovine serum albumin (BSA) and 6 x 10^5 bronchoalveolar leukocytes were placed in the upper compartment and the chambers incubated for 3.5h at 37 ^{0}C. The filters were then removed from the chamber, washed to remove any non-adherent cells and stained with Dif-Kwik (Merz-Dade, Dudingen, Switzerland). Two chambers were set up for each condition and the number of cells that had migrated through the filter was measured in each of ten high power fields per filter.

4.5 Examination of Pulmonary Pathology

All animals in the study were subjected to autopsy. Lung tissue from each was fixed for histological examination together with specimens from any organ showing macroscopic signs of pathological change. With those animals killed at the 12 or 18 months timepoints and for groups of up to 6 animals killed at the termination of the study, one lung was frozen for subsequent dust extraction and analysis with the other being fixed for histology. Both lungs of the remaining animals were processed for histology. The lungs were fixed by inflation with formal saline and embedded in paraffin wax before sectioning for histology. Sections were taken from at least two different levels in the lung and stained with either haemotoxylin and eosin, Van Giessen's method for collagen or Gordon and Sweet's method for reticulin.

4.6 Lung Dust Analysis

For lung dust analysis, either the right or left lung was frozen at autopsy from selected groups of rats. It had been planned that 6 animals from each main killing date would be examined but in the event only 5 animals survived until the final kill from the groups of 18 removed from dusting after 12 months. Rat lungs were individually dried and defatted three times with ether and ethanol mixtures. After drying, the lungs from each experimental batch were bulked together and ground with a mortar and pestle. Aliquots of approximately 150mg were then digested in concentrated hydrochloric acid at 60^{o}C (Rivers *et al.* 1963) to determine the content of non-coal minerals. The residue dust was ashed at either 380^{o}C or 700^{o}C in a muffle asher and aliquots of the ash (0.8mg) were then combined with potassium bromide in discs for mineralogical analysis by infrared spectrophotometry as with the dust samples from the inhalation chambers. Since only single lungs were available for mineralogical analysis, the total mineral content of both lungs was estimated using the factors 1-1:6 between left and right lungs as described previously (Davis *et al.* 1978). To determine coaldust content of lung tissue 150mg aliquots of ground dried lung were digested in 50% potassium hydroxide. The residue dust was then ashed at 380^{o}C in a muffle asher and the coal content was determined by weight loss during this process.

4.7 Statistics

Statistical work on differences in levels of pulmonary fibrosis was undertaken using standard analysis of variance techniques. Differences in levels of short-term pulmonary inflammation were examined using Student's t-test.

5. OBSERVATIONS

5.1 Dust Cloud Calculation and Analysis

Respirable dust clouds were generated on 361 days during the total 18 month inhalation period. Dust concentrations fluctuated from day to day but throughout the exposure period the cumulative mean concentrations were maintained close to the planned 20mg/m^3 mass level with final mean concentration and standard deviations as follows:

	Number of days	Mean respirable dust concentration (and standard deviation)/mg/m^3	Total dust mg/m^3
Scotland	361	19.9 (6.0)	29.4
Nottinghamshire	361	20.0 (4.7)	22.6

The ash and quartz contents of the two dust clouds generated throughout the inhalation period were routinely analysed using incineration at 380°C and infrared absorption spectrophotometry. The figures are given in tables 1–2 and demonstrate good dust composition uniformity throughout the study. Mean ash contents were 75.5% for the Scottish dust and 86.3% for the Nottinghamshire dust. Mean quartz contents were 18.3% for the Scottish dust and 15.7% for the Nottinghamshire dust. In addition the mean kaolinite contents were 25.5% and 18.6% for the Scottish and Nottinghamshire dusts respectively. The illite contents were determined separately in a few samples incinerated at 700°C to reduce the effects of mineral interferences; these illite contents were 17% for the Scottish dust and 32.3% for the Nottinghamshire dust. Determination of illite in 380°C ash is unreliable in the presence of moderate to high amounts of kaolinite. However, measurements of illite were made on the IR spectra routinely prepared for quartz and kaolinite determinations which did demonstrate the consistency of the illite contents and the large differences between the dusts.

In the study by Robertson *et al.* (1984), dusts collected from the same colliery and seam had only 3–4% illite present as determined by IR on 700°C ash.

5.2 Dust Analysis Using Thermoluminescence and Radioluminescence Techniques

These analyses undertaken in the laboratory of Dr W Kriegseis at the University of Giessen, Germany are described in detail in the appendix.

Analysis showed that dust from the Scottish colliery had a much larger free quartz surface than the dust from the Nottinghamshire colliery, by a factor of almost five.

5.3 Histological Examination of Lung Tissue

5.3.1 Animals examined at twelve months

Examination of lung tissue from animals killed after 12 months dusting with the Scottish coalmine dust showed very little pathological change. Dust was of course widespread in the pulmonary parenchyma but most of it was packed into pulmonary macrophages that were widely distributed and at this stage showed little tendency to aggregate together. In a few areas the alveoli contained macrophages

that were much larger and less densely stained than usual and these "foamy" cells contained less dust than the majority of more normal macrophages. Areas where "foamy" macrophages predominated often showed thickening of the alveolar walls with rounding up of epithelial cells, though with as yet no evidence of increased reticulin or collagen staining. Tables 3 and 4 illustrate the extent of areas of interstitial thickening and fibrosis for the whole study and it can be seen that at this stage its occurrence was minimal. Already after 12 months of dusting there had been widespread removal of dust to the hilar and mediastinal lymph nodes. In some areas the dust was densely packed within cells of macrophage type but in others the nodes contained small areas of pale cells of epitheloid type containing relatively little dust and which resembled the quartz typical areas reported by Bruch *et al.* (1975). At this stage of the study there appeared to be no pleural involvement in dust pathology or transport.

Lungs from rats treated with the Nottinghamshire dust for 12 months showed a different pattern. In general much less dust was visible and there were very few macrophages of normal size and staining pattern. Those present appeared to be almost all enlarged and "foamy" and contained only a few dust particles (Figure 3). These cells packed some alveoli but large areas of the lung parenchyma showed widespread alveolar lipoproteinosis with alveoli filled with amorphous pale staining material in which a few dust particles were embedded but with few, if any, macrophages present (Figure 4). Areas of interstitial thickening of alveolar walls were less marked than following treatment with the Scottish dust at this period and certainly there was no tendency for walls to show thickening when alveoli were packed with lipoproteinaceous material. In rats treated with the Nottinghamshire dust for 12 months there had been considerable removal of dust to the lymph nodes and both closely packed dust masses and quartz typical areas were present (Figure 5). Once again the visceral pleura did not appear to be affected at this stage of the study.

5.3.2 Animals examined after eighteen months

Dusting was terminated after 18 months and the lungs of 6 animals dusted for all of this period were examined in addition to the lungs of 4 rats removed from dusting at 12 months and maintained in dust-free conditions for the subsequent 6 months.

Animals treated with the Scottish dust showed no marked increase in pathology although there were some important changes in the distribution of pulmonary macrophages. By now normal-looking pulmonary macrophages, containing considerable amounts of dust, were found far more aggregated in alveoli close to the respiratory and terminal bronchioles and many alveoli in the rest of the lung parenchyma were devoid of dust and cells (Figure 6). This phenomenon was more marked where animals had lived for 6 months without the inhalation of extra dust but it was still noticeable even when dusting had been continued. Some small areas contained "foamy" macrophages with small amounts of dust and these cells showed no tendency to aggregate in the peribronchiolar tissues. Interstitial thickening was again found particularly where "foamy" macrophages were present and though it was by now more marked with greater thickening of alveolar walls, it still involved only a small proportion of lung tissue (Tables 3 and 4).

One important development in both groups of animals treated with the Scottish dust and examined at 18 months was the occurrence of aggregates of dust and cells in the perivascular position of the lung parenchyma forming nodular lesions (Figures 7-10). These nodules consisted of two elements. Most contained a

small mass of lymphocytes but usually the main bulk of the lesions consisted of cells of epitheloid type containing a few dust particles. At this stage the number of nodules was small but some had reached an overall diameter of 0.5mm which is close to the maximum nodule size in older animals. In addition to parenchymal nodules a few small granulomas were found on the viseral pleural surface outside the external elastic lamina of the lung and these were made up of the same cellular elements as the nodules (Figure 11).

Animals examined 18 months after the start of treatment with the Nottinghamshire dust showed the same pattern of changes in those with 18 months continuous exposure as in those that had received 12 months of dusting followed by six months in a dust free environment. The main pathological effect of dusting was widespread alveolar lipoproteinosis with less dust visible than with the Scottish dust and with most recognisable pulmonary macrophages being of the "foamy" type. Those animals which had lived in a dust-free environment for the 6 months not only showed less lipoproteinosis than those inhaling dust all the time but they showed less evidence of this type of pulmonary disease than animals examined immediately after 12 months of dusting. In both groups there was much less evidence of macrophage/dust aggregation in the peribronchiolar region than with the Scottish dust. While areas of interstitial thickening of the alveolar walls was still not common (Tables 3 and 4) some were present and these showed changes not seen at this stage with the Scottish dust. Some of the alveoli in these areas had been filled with cholesterol crystals between which were flattened cells which were probably fibroblasts.

In both populations of rats treated with the Nottinghamshire dust and examined after 18 months there were examples of lymphocyte accumulation in the perivascular position and some clusters of dust-containing macrophages. With this dust, however, these accumulations remained small and did not contain the epitheloid cells that were characteristic of true nodule formation. Small granulomatous lesions were found on the visceral pleural surface as well but although these lesions contained dust and lymphocytes epitheloid cells were sparse.

5.3.3 Animals examined between eighteen months and twenty eight months

A few animals treated with both mine dust samples died between the end of dusting at 18 months and the termination of the study ten months later. These animals were subjected to autopsy and histological examination and demonstrated gradually more severe pulmonary disease. A total of 39 animals survived to the end of the study, 18 of which had been exposed to the Scottish dust and 21 to the Nottinghamshire dust. Of these the numbers exposed for 12 months and for 18 months were 6 and 12 for the Scottish dust and 6 and 15 for the Nottinghamshire dust. With both the mine dust samples the differences in levels of pathology found in the lungs between animals treated for 12 and 18 months were not significantly different (Tables 3 and 4 and section 5.4).

In both groups of animals treated with the Scottish dust, both the development of alveolar interstitial fibrosis and perivascular nodules had increased greatly but with marked variation between animals (Tables 3-5). Some areas of interstitial alveolar wall thickening progressed with little overall change of pattern but with the deposition of much reticulin and some collagen in the interstitial space and with alveoli completely lined with cuboidal epithelial cells. In other areas the normal alveolar structure had been lost but cuboidal epithelial cells still lined tissue spaces giving a pattern of adenomatosis. In yet other cases tissue spaces were filled

with cholesterol crystals with flattened cells between them and in others complete consolidation had occurred. Wall structures were now difficult to recognise and areas of lung tissue consisted of solid masses of granulation tissue cells, often containing foreign body giant cells associated with many dust particles. These areas also contained cholesterol crystals and isolated acinar spaces lined by cuboidal cells (Figures 12–14). While some areas of lung showed almost complete consolidation, however, some showed only lipoproteinosis with alveolar walls appearing relatively normal and many areas showed little signs of pathological change at all.

The most characteristic pathological change in the lungs of animals from the last survivor groups treated with the Scottish dust was the greatly increased profusion of perivascular nodules consisting of lymphocytes and epitheloid cells with dust particles. As shown in Table 5 these could reach a profusion of almost 100/cm^2 of tissue section and their diameters ranged between 0.2mm and 0.5mm. Granulomatous nodules of similar histological pattern to those from the lung parenchyma and containing considerable amounts of dust were frequently found on the visceral pleural surface at this stage. As indicated in Table 5 and section 5.4 there was an inverse relationship between the development of areas of alveolar interstitial change and the profusion of perivascular nodules. An additional lesion of quite separate histological pattern was also present on the visceral pleural surfaces of old animals treated with the Scottish dust. This took the form of a loose mesh of fibrous tissue staining positive for both reticulin and collagen but within which were numerous spaces lined by flattened cells of mesothelial type (Figure 15). This type of lesion has been reported previously in rats treated with asbestos dust (Davis, 1989). However, neither asbestos fibres nor, in the present instance, coalmine dusts are present within the lesion itself and this vescicular fibrosis may be a generalised reaction to widespread tissue damage in the sub-pleural pulmonary parenchyma.

Animals treated with the Nottinghamshire dust for either 12 or 18 months and examined at the end of the study again showed no important differences. Both groups showed continuing widespread alveolar lipoproteinosis to a much greater extent than the animals treated with the Scottish dust. Levels of alveolar interstitial fibrosis leading to complete fibrotic consolidation of some areas of lung tissue had increased greatly since the 18 month stage of the study (Tables 3–5) but apart from less dust being visible in the tissues the patterns of histological change were very similar for both dust types.

The main difference between the pulmonary parenchymal disease caused by the two mine dusts was that there was a complete absence of perivascular nodules in animals treated with the Nottinghamshire dust. In a few cases collections of lymphocytes were present in the perivascular position and these could be associated with macrophages containing dust particles as reported previously. These clusters were perhaps the earliest stages of nodule formation but they lacked the characteristic epitheloid cells of the fully developed nodules and were not counted as nodular lesions for the purposes of comparisons between the dust types. In any case their numbers did not exceed 3–5/sq.cm of lung section compared to 50–100 for the nodules produced for the Scottish mine dust.

As with the Scottish dust treatment, both groups of animals that had inhaled the Nottinghamshire dust and were killed at the end of the study showed widespread vesicular fibrosis on the visceral pleural surfaces and granulomatous nodules containing dust particles were also present. These granulomas were, however, smaller and less frequently found in the animals treated with the Nottinghamshire

dust.

Hilar and mediastinal lymph nodes showed very severe disease in animals from both dust treatments at the end of the study. In most cases the greatly enlarged nodes consisted mainly of fibrosing granulation tissue containing a dense network of reticulin fibres with some of the fibres also staining positive for collagen. Most nodules still contained small areas or columns of lymphocytes but these now made up only a small portion of the total area of these organs.

Two rats from the oldest groups treated with the Nottinghamshire dust had small pulmonary adenomas while 1 animal similarly aged and treated with the Scottish dust also had a lesion of this type. All 3 adenomas had occurred in the centre of large areas of marked interstitial fibrotic thickening and consolidation. Pulmonary adenomas, however, do occur in normal rats of advanced age and these in numbers are similar to those reported previously for the strain of rats used (Davis *et al.* 1986).

5.4 Statistical Analysis of Data on Pulmonary Fibrosis and Formation of Pulmonary Nodules

Figures for levels of interstitial fibrosis in the various groups of animals showed larger variances in the groups with higher mean figures and there was also evidence of skewing in the distributions. For statistical examination, therefore, the data were transformed to logs. The few zero values in the 12 month kills were changed to a value of 0.4 before taking natural logs. When the transformed data were plotted (Figure 16) the variances within each group were approximately equal. The layout of Table 6, and of Figure 16, are designed to facilitate comparisons between the results for animals of similar and different ages when killed, and between animals of the same age who had been exposed to dust for different periods.

For both dusts, the amount of fibrosis present in the animals' lungs clearly increased with time. There was, however, considerable variation between the responses of animals of the same age and exposure history. Against such variation, comparisons of animals killed at the same age after being exposed to the same dust for 12 or 18 months showed no significant differences; thus there was no evidence that exposure for different lengths of time affected the subsequent development of fibrosis. Table 6 includes summary statistics calculated after combining groups of animals killed at the same age.

When the data in Table 6 were subjected to a formal analysis of variance, it was found that more than one model fitted the data equally well. Overall, differences between ages at death were highly significant, and there were also significant differences between the responses to the two dusts, although the large amount of variation between identically treated animals made it difficult to describe with certainty the pattern of those differences. Firstly, a model including only the main effects of age and dust type had a residual sum of squares (rss) of 23.14 on 67 degrees of freedom. This model assumed that the difference between dust types was constant at each age. Because data were considered in the log scale, this implied a constant proportionality in the original scale and the fitted values suggested that dust from the Scottish colliery produced about 41% more fibrosis than the dust from Nottinghamshire. The fitted values from this model are listed in Table 7.

A model which fits a separate mean for each combination of dust and age at kill

had a rss of 22.45 with 65 degrees of freedom. The difference between this and the previous model was not significant. The data, themselves, suggest a model where there is no difference between the dust types at 12 months but an increasing difference at 18 and 28 months estimated as 27% and 67% respectively. This had a rss of 22.45 with 66 degrees of freedom but again was formally not significantly a better fit than the model with only the main effects. Thus any of these three models is as good a description of the patterns in the data as any of the others; the data do not allow us to distinguish between the models. This is a result of the combination of small group size and large within-group variation. However, the overall trend of increasing fibrosis with increasing age was unambiguous.

Pneumoconiotic nodules were only found in any numbers in animals of advanced age treated with dust from the Scottish colliery. Figures were available from two groups dusted either for 12 or 18 months followed by 16 and 10 months in dust-free conditions. All animals in these two groups had some nodules but there was considerable variation in the numbers of nodules observed between animals in the same group and the overall difference between the two groups was not significant. Again, the inability to distinguish between the groups may be due to small group size and large within-group variation.

The relationship between interstitial fibrosis and nodules was investigated for these two groups and the data are summarised in Figure 17. To avoid any possibly inappropriate assumptions of distribution or of linearity, a rank correlation coefficient between the two variables was calculated, which measured the extent to which larger values of one variable tend to occur with larger values of the other, but which ignored the actual sizes of these values. At -0.81 the calculated coefficient was highly significant, confirming the impression that nodules were more prevalent in animals with less fibrosis, and vice versa. Data from both treatment groups were consistent with this trend, although the six values from the 12 month dusting plus 16 month dust-free group would not have been convincing evidence of a relationship on their own.

5.5 Lung Dust Analysis

Examination of the lung dust content of groups of rats treated with mine dusts from collieries in Scotland and Nottinghamshire have confirmed a problem in the extraction of these dusts from tissues recently discovered in our laboratories (unpublished work). When this type of analysis of dust from the lungs of coalminers commenced in our Institute in 1975, the chosen method of dust extraction was concentrated hydrochloric acid (Rivers *et al.* 1963). This method was chosen because it was known that the most usual alternative digestion fluid (potassium hydroxide) caused damage to some of the mineral components of the mine dust particularly the kaolinites. Following extraction by hydrochloric acid the coal content of the dust was determined by weight loss following ashing at 380°C since there is no direct analysis method available for coal. The mineralogical components of the dust were analysed by infrared spectrophotometry. In recent pilot studies, however, it was found that lung dust from rats treated with pure quartz and extracted with hydrochloric acid showed considerable weight loss on ashing. This weight loss obviously resulted from organic tissue residues in this instance but would have been recorded as "coal content" if the rats had been treated with a coalmine dust. It is believed that the organic material causing these problems may well be the lipoprotein exudate that consolidated large areas of the lung tissue of old rats in both experimental groups.

In an attempt to overcome this difficulty, the present study utilised two digestion techniques. Aliquots of dried, triple defatted, powdered rat lung tissue were digested in hydrochloric acid and the residues ashed at 380°C or 700°C before the weight of total ash was determined by weighing and its mineral components by infrared spectrophotometry. Other tissue aliquots were subjected to a two-stage digestion process in 50% potassium hydroxide before the residue was ashed to determine the content of coal (carbonaceous material).

The combined figures from these analyses are illustrated in Tables 8 and 9 with the dust components expressed both by mass and percentage composition. These tables show that even double digestion in potassium hydroxide has failed to remove all organic material from the lungs of old rats where pathological change is at its maximum since the weights of "coal" appear to increase after the end of dusting for both experimental groups while the mineral components fall after the end of dusting and pulmonary clearance begins to reduce the lung dust content. These anomalous "coal" figures to a large extent mask changes in the components of mine dusts following the end of the inhalation period. The best indication of long-term changes in lung dust composition are seen by comparison between the original mine dust composition and that extracted from the rat lungs at the end of the first 12 month inhalation period. At this time point pathological change is relatively slight and the lung content of indigestible carbonaceous material is at its lowest. At this 12 month stage, lung tissue from animals treated with both the mine dust samples shows an increase in the mineral content and a reduction in coal content. This selective retention of minerals has previously been reported from the lungs of coalminers (Ruckley *et al.* 1981). It is likely that selective retention of minerals is progressive and this would have been demonstrated at the later timepoints illustrated in Table 11 if the coal percentage figures had been accurate.

Table 8 shows that at the end of the 12 month inhalation period at 'respirable' mass concentrations which - as measured by the Casella MRE 113A - were almost identical, the amount of dust retained in rat lungs was considerably greater for the rats exposed to Nottinghamshire dust than for those exposed to Scottish dust. The reason is, that the size distributions of the two aerosols were very different, as demonstrated in the relative values of the respirable and total dust concentrations given in section 4.1. The total dust concentration for the Nottinghamshire dust was only 13% higher than the 'respirable' concentration, which implies that relatively little (certainly less than 26%) of the mass distribution exceeded 5μm aerodynamic diameter. By contrast, the total dust concentration for the Scottish dust was 48% higher than the 'respirable' dust concentration. The size selection of particles by the Casella MRE 113A (the BMRC/Johannesburg respirable selection curve) is not the same as that of the human respiratory tract, nor of the rat. Data published by Raabe *et al.* (1988) have shown that the penetration of particles into the rat respiratory tract is much lower over the range 5-7μm aerodynamic diameter than for this gravimetric dust sampler curve. This difference in selection characteristics is sufficient to explain the difference in lung burdens for nominally identical 'respirable dust concentrations'. However, it is also likely that Nottinghamshire dust would have disaggregated (on dispersion into solution) into even finer particles - as observed in the study of Addison *et al* (1990) where it was found that Nottinghamshire dust - collected as aerodynamically separate fractions - dispersed to give fewer coarse particles than in the Scottish dust. It has been demonstrated previously with the rat model that fine dusts penetrate to the pulmonary parenchyma more rapidly than coarse dusts (Davis *et al* 1989).

5.6 Short-term Inhalation Studies: The Development of Pulmonary Inflammation

5.6.1 Numbers of inflammatory cells

The inflammatory response generated by the two mine dusts was markedly different in each of the parameters studied; the biological reactivity of the Nottinghamshire dust was greater than that of the Scottish dust in each case (Table 10, Figure 18). Alveolitis in rats exposed to the Nottinghamshire dust was more rapid in onset and of greater magnitude than in those animals inhaling the Scottish dust. By 15 days after the start of dusting, there was a 3-fold increase in total leukocytes in BAL with the Nottinghamshire dust and by 30 days, the increase was 6-fold compared with control rats. With the Scottish dust the only increase in total BAL leukocytes was found at 30 days of dust exposure when numbers of leukocytes were 3-fold more than in control rats. At the 30 day time point, total leukocytes in rats exposed to the Nottinghamshire dust was almost twice that in those exposed to the Scottish dust. Although numbers of leukocytes in the bronchoalveolar lavage (BAL) of Nottinghamshire dust-exposed rats did not increase significantly until 15 days of exposure, significant recruitment of polymorphonuclear leukocytes (PMN) had occurred by 7 days (Table 11, Figure 19). The % PMN in the BAL of rats exposed to this dust continued to increase with increasing duration of dust exposure. With Scottish dust, PMN recruitment occurred in concert with the increase in total numbers of leukocytes i.e. it was found at 30 days of exposure. At this timepoint there were 3-fold more PMN in the BAL of Nottinghamshire dust-exposed rats than of those inhaling the Scottish coalmine dust.

To assess the effect of cessation of dusting on the lungs of rats exposed to coalmine dust, groups of 4 animals were removed from the chambers at each time point and maintained under normal laboratory conditions for 30 days. During this period, regardless of length of exposure, the total number of lavageable inflammatory cells and the proportion of PMN decreased with both dusts. With the Scottish dust both parameters reached background levels on recovery except following 30 days of exposure when, although total cell numbers returned to control levels, there remained a significant proportion of PMN in the BAL ($p<0.001$ compared with controls). With the Nottinghamshire dust there was full recovery 30 days following 7 days of dust exposure. However, with 15 and 30 days of dust-exposure the inflammatory response persisted throughout the 30 day recovery period. Both total cells and % PMN remained at levels which were significantly greater than controls (Tables 10,11).

5.6.2 Proteolysis of fibronectin

Proteolysis of fibronectin by BAL leukocytes from both groups of dust-exposed rats was unchanged following 3 and 7 days of exposure but at 15 days, the proteolytic activity of BAL leukocytes of rats treated with both coalmine dusts was significantly greater than that of control BAL leukocytes (Table 12). While with fixed cell numbers in the assay, the two dusts showed similar increases in proteolysis, the overall protease burden in the lungs from the Nottinghamshire dust treatment was potentially almost three times greater than that of the Scottish dust treatment since the total number of BAL leukocytes was increased by this amount following exposure to the Nottinghamshire dust. In rats exposed to the Scottish dust there was no persistent increase in fibronectin proteolysis by BAL leukocytes. With the Nottinghamshire dust, however, there was a sustained increase in proteolytic activity after 15 days dusting. No increase in proteolysis on a per cell basis was detected following 30 days of exposure to either dust. The increased leukocyte numbers

present at this timepoint, however, would indicate greatly increased proteolytic activity within the lung tissue.

5.6.3 Chemotaxis

Inhalation of dust reduced the ability of bronchoalveolar leukocytes to migrate along a chemotactic gradient. With Scottish dust, this parameter gave the earliest indication of an effect of dust exposure on the BAL leukocytes. By 7 days of dust exposure there was a significant reduction in chemotaxis with the Scottish dust. No data is available for the Nottinghamshire dust at this timepoint but at both 15 and 30 days of exposure the reduction of chemotaxis was greater in the Nottinghamshire dust-exposed rats (Table 13). Only the Nottinghamshire dust caused a sustained reduction in chemotaxis, in leukocytes from rats recovering from 7, 15 and 30 days of exposure.

6. DISCUSSION

The present study has produced an experimental demonstration of an occurrence that has perplexed researchers and clinicians dealing with the problem of pneumoconiosis in coalmines. A mine dust containing high levels of quartz has produced in the rat model multiple nodules similar in type to early silicotic nodules or the nodules of simple pneumoconiosis in man while another mine dust containing approximately the same levels of quartz has completely failed to produce nodules.

These experimental results are consistent with the human situation since the colliery whose dust produced nodules in rats was mining the same coal seam that had been shown to produce rapidly progressing pneumoconiosis of silicotic type (Seaton *et al.* 1981), while the dust failing to produce nodules in rats came from a colliery with historically very low levels of pneumoconiosis.

It is important to consider whether the combined information obtained from this study helps to explain this phenomenon. In general, research has concentrated on examining the short-term *in vitro* toxicity of mine dusts on the assumption that this would relate to the development of pneumoconiosis but numerous publications have failed to demonstrate a close association. In the present study early pulmonary inflammation was examined and it was found that the dust which eventually failed to produce pneumoconiotic nodules was far more inflammogenic than the one that did. This high toxicity did not correlate with the amount of free quartz surface present in the dust as estimated using thermoluminescence analysis (see appendix).

Le Bouffant (1977) suggested that clay minerals (mica or illite) might release aluminium ions into the pulmonary fluid which could then provide a protective coating to the surface of quartz particles inhaled simultaneously. In this way the toxicity of coalmine dusts might not depend directly upon the quartz content of individual mine dusts.

The two dusts examined in the present study were in fact chosen to examine this hypothesis with dusts from the real mining situation. The dust from the Scottish colliery was known to contain much less of the mica/illite than the dust from the Nottinghamshire colliery. As far as short-term dust toxicity is concerned, a protective effect was certainly not demonstrated, with the Nottinghamshire dust producing far more widespread pulmonary inflammation than the Scottish dust with a major influx of both pulmonary macrophages and neutrophil leukocytes to the lung tissue and subsequently widespread alveolar lipoproteinosis.

Combined evidence from the present study and a previous report by Robertson *et al.* (1984) also demonstrates that the development of pneumoconiosis is unaffected by a wide range of illite concentrations at least within one colliery. In the 1984 report, it was shown that rats treated with dust from a Scottish colliery having high quartz levels but only 3-4% illite, developed nodular pneumoconiosis. In the present study, dust containing similar amounts of quartz produced similar pneumoconiotic lesions in rats although the illite levels had risen to 17%. The Nottinghamshire dust contains a certain amount of mixed layer illite/smectite which may not be as abundant in the Scottish dust. However, while these clay mineral types might release more alkali elements into the pulmonary fluids, these elements are already present in large amounts and there is no indication of any greater ability on the part of these minerals to provide aluminium to solution or any

protective effect.

It is known from the geological descriptions of the non-coal rocks forming the roof and floor to the seams as well as the mid-seam horizons that the major sources of the quartz in the two coalmines are very different (Sinha and Annis, 1977; Seaton *et al.* 1981). The Nottinghamshire dust is generated mostly from shales and mudstones in which the intrinsic particle sizes are of the order of 1 to 2μm. The quartz particles among these will have naturally coated ancient surfaces derived from percolating ground waters and epithermal solutions. It is reasonable to suggest that these quartz particles are unlikely to be heavily fractured during coalmining rather than simply separated along grain boundaries from other small particles. In contrast, the quartz in the Scottish mine dust is derived mostly from a medium to coarse-grained sandstones in the roof and floor of the seam. The intrinsic quartz particle diameter in those rocks is probably greater than 250μm and it is very likely therefore that the fine respirable quartz of the Scottish dust consists of freshly fractured fragments of the large quartz grains. It is similarly likely that the surfaces of these respirable quartz particles would be free from contamination of any type at the time of their inhalation.

This geological information coincides with the data provided from the radioluminescence and thermoluminescence tests carried out by Dr Kreigseis which found a much greater free quartz surface area in the Scottish dust than in the Nottinghamshire dust. However, larger free quartz surface would normally be associated with a finer particle size (for the same amount of quartz) so it is interesting that Addison (1990) indicated that the finer grained quartz was present in the Nottinghamshire dusts which actually had the smaller free quartz surface area. This would appear to be possible only if the quartz in the Nottinghamshire dust had a heavily contaminated surface as suggested by the geological origins.

It may be that the more marked inflammatory response generated by the Nottinghamshire dust is a result of greater particle numbers and finer particle sizes among all the minerals while the nodular silicotic response generated by the Scottish dust is a result of highly active free-quartz surfaces in the dust.

The possible biological reasons why the development of pneumoconiotic nodules does not relate to short-term toxicity as seen in experimental studies are discussed later but from the clinical point of view, this lack of relationship need not be surprising. It must be remembered that the early diagnosis of pneumoconiosis in coalminers depends almost entirely on the occurrence of pulmonary nodules large enough to produce small opacities on chest radiographs and that it is accepted that simple pneumoconiosis does not cause loss of pulmonary function. The condition of acute silicosis is of course recognised, where exposure to dust with very high quartz levels is excessive and the pathology of this condition is very similar to that seen in the rat model with inflammation and lipoproteinosis predominating. With the relatively low dust levels in modern collieries, however, and with dusts containing rarely more than 20% of quartz and usually much less, short-term toxic effects on the lung tissue may still occur to varying degrees but may not produce recognisable clinical symptoms, which could later be correlated to the development of pneumoconiosis.

In this study the short-term inflammatory response to the two dusts in the bronchoalveolar region was very different, with the Scottish dust producing little immediate inflammation. Interstitial fibrosis was observed in the long-term in animals from both groups, with the Scottish dust producing about 67% more than the Nottinghamshire dust by the end of the experiment. The most marked

difference in pathology between the two dusts was the presence of numerous nodules at the level of the terminal bronchioles and these occurred only with the Scottish dust which was least inflammogenic in the short term. Thus, although the pathological findings correlated with human epidemiology data, the short-term inflammatory data did not fit with previous experimental data on the pathogenesis of lung fibrosis. In other studies using the rat model it has been shown that the magnitude of the inflammatory bronchoalveolar response to inhaled particles is directly related to the fibrogenic potential of the dust (Donaldson *et al.* 1988; Brown and Donaldson, 1989). In human studies the presence of coalworkers pneumoconiosis is also associated with the presence of inflammatory leukocytes in the bronchoalveolar region (Sablonniere *et al.* 1983; Voisin *et al.* 1985; Begin *et al.* 1986). What is not known is how the magnitude of the inflammatory response is related to the development of pneumoconiosis in coalworkers.

Intrinsic to an understanding of how coalmine dust exposure leads to nodule formation is knowledge of how lung inflammation and inflammatory leukocyte function may be modulated by different dusts and so contribute to the formation of the pneumoconiotic nodules.

The two dusts used in this study contained similar high levels of quartz, a dust which is markedly inflammogenic in experimental studies of the pure mineral and which may therefore be expected to contribute the major element of the inflammogenic properties of the two dusts. It is widely accepted and has been extensively demonstrated experimentally that the inflammatory response to silica is related to the free quartz surface (Begin *et al.* 1987; Brown and Donaldson and Brown, 1989; Wiessner *et al.* 1990). In the present study, however, the Scottish dust which had the greatest free quartz surface, caused the least inflammatory response. Conversely, the Nottinghamshire dust had a low level of free quartz surface but produced an inflammatory response which was very similar to that elicited by exposure to pure quartz in man (Seaton *et al.* 1987) and in previous experimental studies (Begin *et al.* 1987; Brown and Donaldson, 1989; Wiessner *et al.* 1990), i.e. marked alveolitis and lipoproteinosis. One explanation for this inconsistency may be differences in the definition of free quartz surface. In the present study, free quartz surface was defined by parameters of activity in thermoluminescence and radioluminescence assays, whereas in studies measuring the biological reactivity of quartz, surface-modified silica has been compared with untreated silica (Begin *et al.* 1987; Brown and Donaldson, 1990; Wiessner *et al.* 1990).

The unexpectedly low inflammogenicity of the Scottish dust may be due to coating of the quartz surface with lipid components of the lung lining fluid. Quartz coated with lipids which are representative of those present in the bronchoalveolar region is not toxic to cells *in vitro* until these components are leached off the dust in the lysosomes (Wallace *et al.* 1989). If the type of quartz in the two dusts in this study differed in ability to retain bound lipids, then differential inflammogenicity might also be predicted.

The various phospholipids which compose the lung lining fluid may also have different properties in modulating the harmfulness of silica. The proportions of the individual lipids in BAL fluid can vary which suggests that their secretion by epithelial cells may be differentially inducable. Alterations can occur in the ratio of phosphatidyl glycerol and phosphatidyl inositol to immunoglobulin G in BAL fluid, for example, and can change surfactant properties from suppressive to stimulatory (Jabbour *et al.* 1991). It is possible, therefore, that differences between the two dusts were mediated by their effect on the epithelial cells to

cause differential secretion of surfactant molecules and subsequent modulation of the inflammatory response.

Although there was a markedly more intense inflammation in the bronchoalveolar region in rats exposed to the Nottinghamshire coalmine dust, with greater potential for proteolytic injury and repair to occur, the Scottish dust produced greater amounts of interstitial fibrosis and was the only one to produce pneumoconiotic nodules. These results are in contradiction with previous theories of the pathogenesis of fibrotic lung disease. However, recent work by McGavran *et al.* 1990 has indicated that cell proliferation, which may lead on to fibrotic change, can occur in the alveolar region in response to inhaled dust in the absence of an inflammatory response. In addition, fibrotic lesions are known to arise in the lung parenchyma in neutropenic subjects (Maunder *et al.* 1986). Taken together, these studies suggest that different dusts may act to produce fibrosis via different mechanisms and those mechanisms need not necessarily involve overt lung inflammation.

The difference in the type of pathology observed with these two dusts may be related to the functional activity of the lavage leukocytes. An important factor contributing to the persistence of the inflammation may be the reduced chemotaxis of the bronchoalveolar leukocytes. There was a more marked reduction in the chemotactic activity of bronchoalveolar leukocytes from rats exposed to Nottinghamshire coalmine dust than in those exposed to the Scottish dust. The greater amount of dust in the lungs of the former at the end of dusting might be due to reduced clearance caused by the impaired ability of the leukocytes to migrate. With the Scottish dust, where the reduction in chemotaxis was less marked, clearance of dust via the lymphatics and possibly by transfer into the interstitium, might contribute to the development of nodules. The occurrence of aggregations of dust-laden macrophages close to the terminal and respiratory bronchioles supports the hypothesis that accumulation of dust-laden macrophages at this site, where the lymphatic system arises in the deep lung, may be a contributory factor in nodule development.

Measuring the bronchoalveolar inflammation takes account only of events in the airspaces of the lung and does not address the interstitial leukocyte response. The presence of dust in the lung causes a greater fibrotic response if the epithelium is compromised, so permitting the dust to migrate to the interstitium (Adamson *et al.* 1989). It is possible that much of the Scottish dust was transported to the interstitium and the injury occurred at that site while the bronchoalveolitis in the rats exposed to the Nottinghamshire dust would cause injury mainly while the dust particles were free in the airspaces.

Robertson *et al.* (1984) and Vincent *et al.* (1987) demonstrated that when mixtures of coal and quartz are inhaled by rats, quartz is preferentially removed to the hilar and mediastinal lymph nodes. Robertson *et al.* (1984) and Davis *et al.* (1991) reported that clearance of quartz-containing dusts (either coalmine dusts or artificial mixtures of asbestos and quartz) from rat lung could result in the formation of granulomatous nodules on the visceral pleural surface outside the pulmonary external elastic lamina as well as similar nodules in the pulmonary parenchyma. These pulmonary granulomas could contain both coal dusts or asbestos fibres depending on the dust mixtures used but these dusts when inhaled on their own did not penetrate the pleural surface. These findings suggest that the lymphatic transport of quartz also facilitates transport of other dust particles. That the speed and extent of lymphatic transport of dusts could relate to the production of pulmonary nodules was suggested by Bruch *et al.* (1975) who reported that the

appearance and extent of "quartz typical areas" (QTA) in rats treated with coalmine dusts showed good correlation with the prevalence of pneumoconiosis in the collieries from which the dusts were obtained.

In the present study the mass levels of dust in pulmonary lymph nodes were not measured. Although 'Quartz typical areas' (QTAs) were certainly common in lymph nodes from animals treated with dusts from the Scottish and Nottinghamshire collieries at the end of a 12 month dusting period, this timepoint was too late to examine their early development.

In the present study although the respirable mass concentrations of quartz were similar in the dust clouds, only the Scottish dust produced nodules. If these nodules are a result of exposure to the freshly fractured quartz surfaces in the Scottish dust, it must be concluded that the long-term reaction to inhaled quartz in the rat or human lung will be dependent on the source and nature of the quartz, and not solely on the amount present. This has important consequences for setting occupational exposure limits (OELs) for quartz in mixed dusts. For example, had information on the incidence of pneumoconiosis in Nottinghamshire collieries been used as data for setting an OEL for quartz, the protection offered to workers in the Scottish colliery would have been inadequate. Conversely if data from the Scottish colliery had been used to set a universal OEL for quartz, this standard might have been inappropriately stringent for the Nottinghamshire industry, and indeed might not have been practical. The problem in setting OELs, therefore, is that in a given situation it may not be possible to deduce from measurements of dust or quartz mass concentration the likely risk of pneumoconiosis presented to the workforce.

None of these results provides us with any information about the problem of coalworkers' pneumoconiosis in the collieries of South Wales. While we believe the pneumoconiotic appearances as a result of exposure to the Scottish dust are similar to those of stoneworkers' silicosis (Seaton *et al.* 1981), these appearances differ from those in affected workers from South Wales, who were exposed to anthracite dusts containing very little quartz. While the rat inhalation model has produced results for quartz in coalmine dust consistent with human epidemiology, we do not know how the rat lung would respond in the long-term to the inhalation of anthracite dusts from South Wales.

7. CONCLUSIONS

1. In the rat lung, inhalation of the Scottish dust was followed by the formation of fibrotic nodules similar to those of pneumoconiosis or silicosis, while the Nottinghamshire dust failed to produce nodules. These results are entirely consistent with previous inhalation studies of a similar Scottish dust and with findings from human epidemiology, confirming that the differences observed in human populations from different collieries or coalfields are likely to result from differences in the dusts to which they exposed rather than to other, e.g. geographical or environmental, differences.

2. The Scottish dust contained around 17% illite, from which it can be concluded that the presence of illite in general does not prevent the development of pneumoconiosis.

3. Although the respirable quartz mass concentrations in the inhalation chambers were similar and although the Nottinghamshire dust was finer and thus of greater surface area, the measured free quartz area of the Scottish dust was very much greater, giving strong support to the view that the pathogenicity of quartz increases with its free quartz area. These results have implications for the setting of occupational exposure limits, since they imply that the risk of developing radiological change is a function not only of the amount of quartz present, but also of its type or nature.

4. The Nottinghamshire dust produced a pronounced inflammatory response following short-term exposure, and diffuse alveolar lipoproteinosis after prolonged exposure. These results show that the presence of such inflammatory responses is not necessarily a useful guide to the risk of development of pneumoconiosis. Inflammation and the formation of nodules may be separate processes; further work is necessary to examine the cellular mechanisms involved, and how they relate to each other.

5. The agreement between the formation of fibrotic nodules in the rat lung and radiological change in coalminers suggests that there may be a useful role for rat inhalation studies to study the ability of other dusts to produce pneumoconiotic changes. Further work should include comparisons with dusts such an anthracites, which are known to carry a high risk of pneumoconiosis although they typically contain little quartz. If the responses over a range of dusts are consistent with results from human epidemiology, it is conceivable that rat inhalation studies could be used in future to assess risks from dusts created in new or changed mining conditions.

REFERENCES

Adamson IYR, Letourneau HL, Bowden DH (1989) Enhanced macrophage-fibroblast interactions in the pulmonary interstitium increases fibrosis after silica injection to monocyte depleted mice. American Journal of Pathology; 134: 411-418

Addison J, Bolton RE, Davis JMG, Dodgson J, Gormley IP, Hadden GG, Robertson A (1982) The relationship between epidemiological data and the toxicity of coalmine dusts. Edinburgh: Institute of Occupational Medicine. (IOM Report TM/82/22)

Addison J, Miller BG, Porteous RA, Dodgson J, Robertson A (1990) . Shape, size and composition of coalmine dusts in relation to pneumoconiosis in British coalmines. Edinburgh: Institute of Occupational Medicine. (IOM Report TM/90/10)

Attygale D, King EJ, Harrison CV, Nagelschmidt G (1956) The action of variable amounts of tridymite, and of tridymite combined with coal, on the lungs of rats. British Journal of Industrial Medicine; 13: 41-50

Beckett ST (1975) The generation and evaluation of UICC asbestos clouds in animal exposure chambers. Annals of Occupational Hygiene; 18: 187-198

Begin R, Bisson G, Boileau R, Massé S (1986) Assessment of disease activity by Gallium-67 scan and lung lavage in the pneumoconiosis. Seminars in Respiratory Medicine; 7: 271-280

Begin R, Massé S, Sebastien P, Martel M, Bossé J, Dubois F, Geoffroy M, Labbé J (1987) Sustained efficacy of aluminium to reduce quartz toxicity in the lung. Experimental Lung Research; 13: 205-222

Belt TH, King EJ (1945) Tissue reactions produced experimentally by selected dusts from South Wales coalmines. In: Medical Research Council. Chronic pulmonary disease in South Wales coalmines III. Experimental studies. London: HM Stationery Office: 29-68

Bingham E, Barkley W, Murphy R, Vassallo C (1977) Investigation of alveolar macrophages from rats exposed to coal dust. In: Walton WH, ed. Inhaled particles IV. vol 2. Oxford: Pergamon 543-550

Borm PJA, Palmen N, Engelen JM, Buurman WA (1988) Spontaneous and stimulated release of tumour necrosis factor-alpha (TNF) from blood monocytes of miners with coalworkers' pneumoconiosis. American Review of Respiratory Disease; 138: 1589-1594

Brown GM, Donaldson K (1988) Degradation of connective tissue components by lung-derived leukocytes *in vitro*: role of proteases and oxidants. Thorax; 43: 132-139

Brown GM, Donaldson K (1989) Inflammatory responses in lungs of rats inhaling coalmine dust: enhanced proteolysis of fibronectin by bronchoalveolar leukocytes. British Journal of Industrial Medicine; 46: 866-872

Brown GM, Donaldson K, Brown D (1989) Bronchoalveolar leukocyte response in experimental silicosis modified by soluble aluminium compounds. Toxicology and Applied Pharmacology; 101: 95-105

Bruch J, Karim A, Kramer U, Hilscher W (1975) Determinations by means of Digiscan phase integrator concerning dust deposits and dust distributions of histological sections of lymph nodes of rats subsequent to intraperitoneal injection of different mine dusts. In: Ergebnisse von Untersuchungen auf dem Gebiet der Staub- und Silikosebekampfung im Steinkohlenbergbau; 10: 173-176

Bruch J, Joos MR, Reisner MTR, Rosmanith J (1983) Untersuchungen zur spezifischen Schädlichkeit von Feinstauben des Steinkohlenbergbaus XVII. Vergleich der Ergebnisse von Tier und Zellversuchen. In: Ergebnisse von Untersuchungen auf dem Gebiet der Staub- und Silikosebekampfung im Steinkohlenbergbau; 14: 241-255

Casswell C, Bergman I, Rossiter CE (1971) The relation of radiological appearance of simple coalworkers' pneumoconiosis to the content and composition of the lung. In: Walton WH, ed. Inhaled particles III. Old Woking, Surrey: Unwin Bros. 713-724

Crystal RG, Gadek JE, Victor J, Ferrans MD (1981) Interstitial lung disease: current concepts of pathogenesis, staging and therapy. The American Journal of Medicine; 70: 542-569

D'Arcy Hart P, Aslett EA (1942) Chronic pulmonary disease in South Wales coalminers. (MRC Special Report Series No. 243). London: Medical Research Council.

Davis JMG, Beckett ST, Bolton RE, Collings P, Middleton AP (1978) Mass and number of fibres in the pathogenesis of asbestos-related lung disease in rats. British Journal of Cancer; 37: 673-688

Davis JMG, Chapman J, Collings P, Douglas AN, Fernie J, Lamb D, Ottery J, Ruckley VA (1979) Autopsy studies of coalminers' lungs. Edinburgh: Institute of Occupational Medicine. (IOM Report TM/79/9)

Davis JMG, Addison J, Bolton RE, Donaldson K, Jones AD, Smith T (1986) The pathogenicity of long versus short fibre samples of amosite asbestos administered to rats by inhalation and intraperitoneal injection. British Journal of Experimental Pathology; 67: 415-430

Davis JMG (1989) Mineral fibre carcinogenesis: experimental data relating to the importance of fibre type, size, deposition, dissolution and migration. In: Bignon J, Peto J, Saracci R, eds. Non-occupational exposure to mineral fibres. Lyon: International Agency for Research on Cancer pp.33-46. (IARC Publication No. 90)

Davis JMG, Jones AD, Miller BG (In press) Experimental studies in rats on the effects of asbestos inhalation coupled with the inhalation of titanium dioxide or quartz.

Dodgson J, Whittaker W (1973) The determination of quartz in respirable dust samples by infrared spectrophotometry - I. The potassium bromide disc method. Annals of Occupational Hygiene; 16: 373-387

Donaldson K, Bolton RE, Jones AD (1988) Kinetics of bronchoalveolar response in rats following exposure to equal airborne mass concentrations of quartz, chrysotile asbestos or titanium dioxide. Thorax; 43: 525–533

Donaldson K, Brown GM, Brown DM, Robertson MD, Slight J, Cowie H, Jones AD, Bolton RE, Davis JMG (1990[a]) Contrasting bronchoalveolar leukocyte responses in rats inhaling coalmine dust, quartz or titanium dioxide: effects of coal rank, airborne mass concentration and cessation of exposure. Environmental Research; 52: 62–76

Donaldson K, Brown GM, Brown DM, Slight J, Robertson MD, Davis JMG (1990[b]) Impaired chemotactic responses of bronchoalveolar leukocytes in experimental pneumoconiosis. Journal of Pathology; 160: 63–69

Donaldson K, Slight J, Brown DM (1989) Effects of products from inflammatory pulmonary neutrophils on alveolar macrophage chemotaxis, spreading and thymidine incorporation. Inflammation; 13: 443–453

Einbrodt HJ (1965) Quantitative und qualitative Untersuchungen über die Staubretention in der menschlichen Lunge. Beiträge Silikose-Forschung; 87.

Faulds JS, King EJ, Nagelschmidt G (1959) The dust content of the lungs of coalworkers from Cumberland. British Journal of Industrial Medicine; 16: 43–50

Gormley IP, Collings P, Davis JMG, Ottery J (1979) An investigation into the cytotoxicity of respirable dusts from British collieries. British Journal of Experimental Pathology; 60: 526–535

Gough J, James WRL, Wentworth JE (1949) Comparisons of the radiological and pathological changes in CWP. Journal of the Faculty of Radiologists (London); 1: 28–39

Heppleston AG (1947) Essential lesions of pneumoconiosis in Welsh coal trimmers. Journal of Pathology and Bacteriology; 59: 453–460

Heppleston AG (1951) Coalworkers pneumoconiosis. Archives of Industrial Hygiene and Occupational Medicine; 4: 270–288

Heppleston AG (1953) The pathological anatomy of simple pneumoconiosis in coalworkers. Journal of Pathology and Bacteriology; 66: 235–246

Heppleston AG (1954) The pathogenesis of simple pneumoconiosis in coalworkers. Journal of Pathology and Bacteriology; 67: 51–63

Hunninghake GW, Garrett KC, Richerson HB, Fantone JC, Ward PA, Rennard SJ, Bitterman PB, Crystal RG (1984) Pathogenesis of granulomatous lung diseases. American Review of Respiratory Disease; 130: 476–496

Hurley JF, Burns J, Copland L, Dodgson J, Jacobsen M (1982) Coalworkers' simple pneumoconiosis and exposure to dust at ten British coalmines. British Journal of Industrial Medicine; 39: 120–127

Jabbour AJ, Holian A, Scheule RK (1991) Modification of chrysotile (CHR) asbestos bioactivity by lung lining fluid (LCF). American Review of Respiratory Disease; 143: A267

Jacobsen M, Rae S, Walton WH, Rogan JM (1971) The relation between pneumoconiosis and dust exposure in British coalmines. In: Walton WH, ed. Inhaled particles III. Old Woking, (Surrey): Unwin Bros 903-917

Jacobsen M, Maclaren WM (1982) Unusual pulmonary observations and exposure to coalmine dust: a case-control study. In: Walton WH, ed. Inhaled particles V. Oxford: Pergamon: 753-765

Kaplan A (1962) Correlation of radiological category with lung pathology in CWP. British Journal of Industrial Medicine; 19: 171-179

King EJ, Nagelschmidt G (1945) The mineral content of the lungs of workers from the South Wales coalfield. In: Medical Research Council. Chronic pulmonary disease in south Wales coalminers. III. Experimental studies. London: HM Stationery Office; 1-20 (MRC Special Report Series no. 250).

King EJ, Maguire BA, Nagelschmidt G (1956) Further studies of the dust in lungs of coalminers. British Journal of Industrial Medicine; 13: 9-23

Kriegseis W, Biederbick R, Boese J, Robock K, Scharmann A (1977) Investigations into the determination of the cytotoxicity of quartz dust by physical methods. In: Walton WH, ed. Inhaled particles IV. Oxford: Pergamon: 345-359

Kriegseis W, Scharmann A (1982) Specific harmfulness of respirable dusts from West German coalmines. Annals of Occupational Hygiene; 26: 511-527

Kuhn DL (1990) Effect of *in vivo* coal dust exposure on arachidonic acid metabolism in the rat alveolar macrophage. Journal of Toxicology and Environmental Health; 29: 157-168

Le Bouffant L, Daniel H, Martin JC (1977) The therapeutic action of aluminium compounds on the development of experimental lesions produced by pure quartz or mixed dust. In: Walton WH, ed. Inhaled particles IV. Oxford: Pergamon: 389-400

Le Bouffant L, Daniel H, Martin JC, Aubin C, Le Heuede P (1983) Recherche communautaire sur le role de quartz dans la pneumoconiose des mineurs de charbon et sur l'influence des mineraux d'accompagnement. Verneuil-en-Halatte: Cerchar

Le Bouffant L (1988) Community research into the relationship between epidemiological data and the toxicity of mine dusts. Commission of the European Communities, Luxembourg: EUR 10872

Maunder RJ, Hackman RC, Riff E, Albert RK, Springmeyer SC (1986) Occurrence of adult respiratory distress syndrome in neutropenic patients. American Review of Respiratory Disease; 133: 313-316

McGauran PD, Moore LB, Brody AR (1990) Inhalation of chrysotile asbestos induces rapid cellular proliferation in small pulmonary vessels of rats and mice. American Journal of Pathology; 136: 695–205

Miller BG, Kinnear AG (1988) Pneumoconiosis in coalminers and exposure to dust of variable quartz content. Edinburgh: Institute of Occupational Medicine. (IOM Report TM/88/17)

Naeye R, Mahon JK, Dellinger WS (1971) Rank of coal and coalworkers pneumoconiosis. American Review of Respiratory Disease; 103: 350–355
Naeye R, Dellinger WS (1972) Coalworkers' pneumoconiosis: correlation of roentgenographic and post mortem findings. Journal of the American Medical Association; 220: 223–227

Nagelschmidt G, Rivers D, King EJ, Trevella W (1963) Dust and collagen content of lungs of coalworkers with progressive massive fibrosis. British Journal of Industrial Medicine; 20: 181–191

Orenstein AJ (1960) Proceedings of Pneumoconiosis Conference, Johannesburg 1959. London: Churchill.

Raabe OG, Al-Bayati MA, Teague SV, Rasolt A (1988) Regional deposition of inhaled monodisperse coarse and fine aerosol in small laboratory animals. In: McCallum RI, ed. Inhaled particles V. Oxford: Pergamon 53–64

Reisner MTR (1971) Results of epidemiological studies of pneumoconiosis in West German coalminers. In: Walton WH, ed. Inhaled particles III
Unwin Bros: Old Woking, (Surrey): 921–931

Reisner MTR, Robock K (1977) Results of epidemiological, mineralogical and cytotoxicological studies on the pathogenicity of coalmine dusts. In: Walton WH, ed. Inhaled particles IV. Oxford: Pergamon: 703–715

Reisner MTR (1983) Untersuchung der Beziehungen zwischen den epidemiologische Ergebnissen und der Schädlichkeit der Feinstaube im Kohlenbergbau. Essen: Bergbau–Forschung GmbH.

Rivers D, Wise ME, King EJ, Nagelschmidt G (1960) Dust content, radiology and pathology in simple pneumoconiosis of coalworkers. British Journal of Industrial Medicine; 17: 87–108

Rivers D, Morris TG, Wise ME, Cooke TH, Roberts WH (1963) The fibrogenicity of some respirable dusts measured in mice. British Journal of Industrial Medicine; 20: 13–23

Robertson A, Bolton RE, Chapman J, Davis JMG, Dodgson J, Gormley IP, Jones AD, Miller B (1984) Animal inhalation experiments to investigate the significance of high and low percentage concentrations of quartz in coalmine dusts in relation to epidemiology and other biological tests. Edinburgh: Institute of Occupational Medicine. (IOM Report TM/84/5).

Rom WN, Bitterman PB, Rennard SJ, Cantin A, Crystal RG (1987) Characterisation of the lower respiratory tract inflammation of nonsmoking individuals with chronic inhalation of inorganic dusts. American Review of Respiratory Disease; 136: 1429–1434

Rossiter CE, Rivers D, Bergman I, Casswell C, Nagelschmidt D (1967) Dust content, radiology and pathology in simple pneumoconiosis of coalworkers. In: Davies CN, ed. Inhaled particles and vapours II. Oxford: Pergamon: 419-437

Rossiter CE (1972) Relation between content and composition of coalworkers lungs and radiological appearances. British Journal of Industrial Medicine; 29: 31-44

Ruckley VA, Chapman JS, Collings P, Douglas AN, Fernie JM, Lamb D, Davis JMG (1981) Autopsy studies of coalminers lungs. Phase III. Edinburgh: Institute of Occupational Medicine. (IOM Report TM/81/18)

Ryder R, Lyons JP, Campbell H, Gough J (1970) Emphysema and coalworkers' pneumoconiosis. British Medical Journal; 3: 481-487

Sabloniere B, Scharfman A, Lafitte JJ, Laine A, Aerts C, Hayem A (1983) Enzymatic activities of bronchoalveolar lavages in coalworkers' pneumoconiosis. Lung; 161: 219-228

Schlipkoter HW, Hilscher W, Pott F, Beck EG (1971) Investigations on the aetiology of coalworkers' pneumoconiosis with the use of PVN-oxide. In: Walton WH, ed. Inhaled particles III. Old Woking, (Surrey): Unwin Bros: 379-390

Seaton A, Dick JA, Dodgson J, Jacobsen M (1981) Quartz and pneumoconiosis in coalminers. Lancet; ii:1271-1275

Seaton A, Addison J, Davis JMG, Hurley JF, McGovern B, Miller BG (1987) The toxic effects of silica. Edinburgh: Institute of Occupational Medicine. (IOM Report TM/87/13)

Sinha MM, Annis R (1977) Pneumoconiosis Field Research: environmental conditions at Linby Colliery. Edinburgh: Institute of Occupational Medicine. (IOM Report TM/77/9)

Spink R, Nagelschmidt G (1963) Dust and fibrosis in the lungs of coalworkers from the Wigan area of Lancashire. British Journal of Industrial Medicine; 20: 118-123

Sutherland C (1945) Section on radiology. Discussion on modern conceptions of industrial lung diseases. Proceedings of the Royal Society of Medicine; 38: 519-534

Sweet DW, Crouse WE, Crable JV, Carlberg JF, Lainhart WS (1974) The relationship of total dust-free silica and trace metal concentrations to the occupational respiratory disease of bituminous coalminers. American Industrial Hygiene Association Journal; 35: 479-488

Vincent JH, Jones AD, Johnston AM, McMillan C, Bolton RE, Cowie H (1987) Accumulation of inhaled mineral dust in the lung and associated lymph nodes: implications for exposure and dose in occupational lung disease. Annals of Occupational Hygiene; 31: 375-393

Voisin C, Wallaert B, Aerts C, Brosbois JM (1985) Bronchoalveolar lavage in coalworkers' pneumoconiosis: oxidant and antioxidant activities of alveolar macrophages. In: Beck EG, Bignon J, eds. *In vitro* effects of mineral dusts. Berlin: Springer Verlag 93-100

Vyskocil J, Tume J, Macek M (1970) Relations between morphological changes and content of silica and hydroxyproline in bronchi and lungs of coalminers. International Archiv für Arbeitsmedizin; 26: 157-166

Wallace WE, Keane MJ, Mike PS, Hill CA, Vallyathan V (1989) Mineral surface-specific differences in the adsorption and enzymatic removal of surfactant and their correlation with cytotoxicity. In: Mossman BT, Begin RO, eds. Effects of mineral dusts on cells. Berlin: Springer Verlag 37-49

Walton WH, Dodgson J, Hadden GG, Jacobsen M (1977) The effects of quartz and other non-coal dusts in coalworkers' pneumoconiosis. In: Walton WH, ed. Inhaled particles IV. Oxford: Pergamon: 669-690

Weller W, Rosmanith J (1983) Untersuchungen zur spezifischen Schädlichkeit von Feinstauben des Steinkohlenbergbaus. XVIII Vergleich der Wirkung eines grubenechten Staubes im Intraperitonealtest, im Intratrachealtest und Inhalationstest. In: Ergebnisse von Untersuchungen auf dem Gebiet der Staub- und Silikosebekampfung im Steinkohlenbergbau; 14: 257-264

Wiessner JH, Mandel NS, Sohnle PG, Hasegawa A, Mandel GS (1990) The effect of chemical modification of quartz surfaces on particulate-induced pulmonary inflammation and fibrosis in the mouse. American Review of Respiratory Disease; 141: 111-116

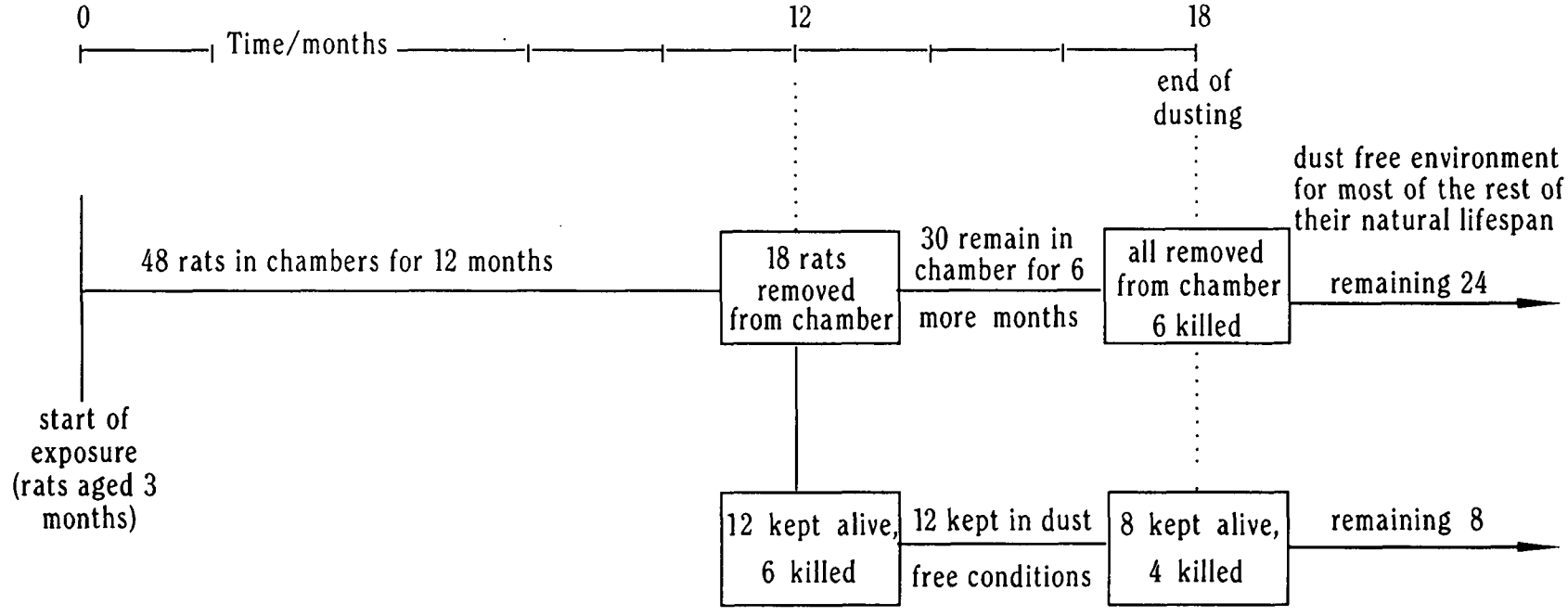
Worth G, Muysers von K, Einbrodt HJ (1968) Über die Korrelationein von röntgenologischen, pathologisch-anatomischen und staubanalytischen Befunden bei der Kohlenbergarbeiterpneumokoniose. Beitrage zur Silikoseforschung: 96: 1

Wright BM (1950) A new dust feed mechanism. Journal of Scientific Instruments; 27: 12-15

Figure 1

Plan of exposure regimen

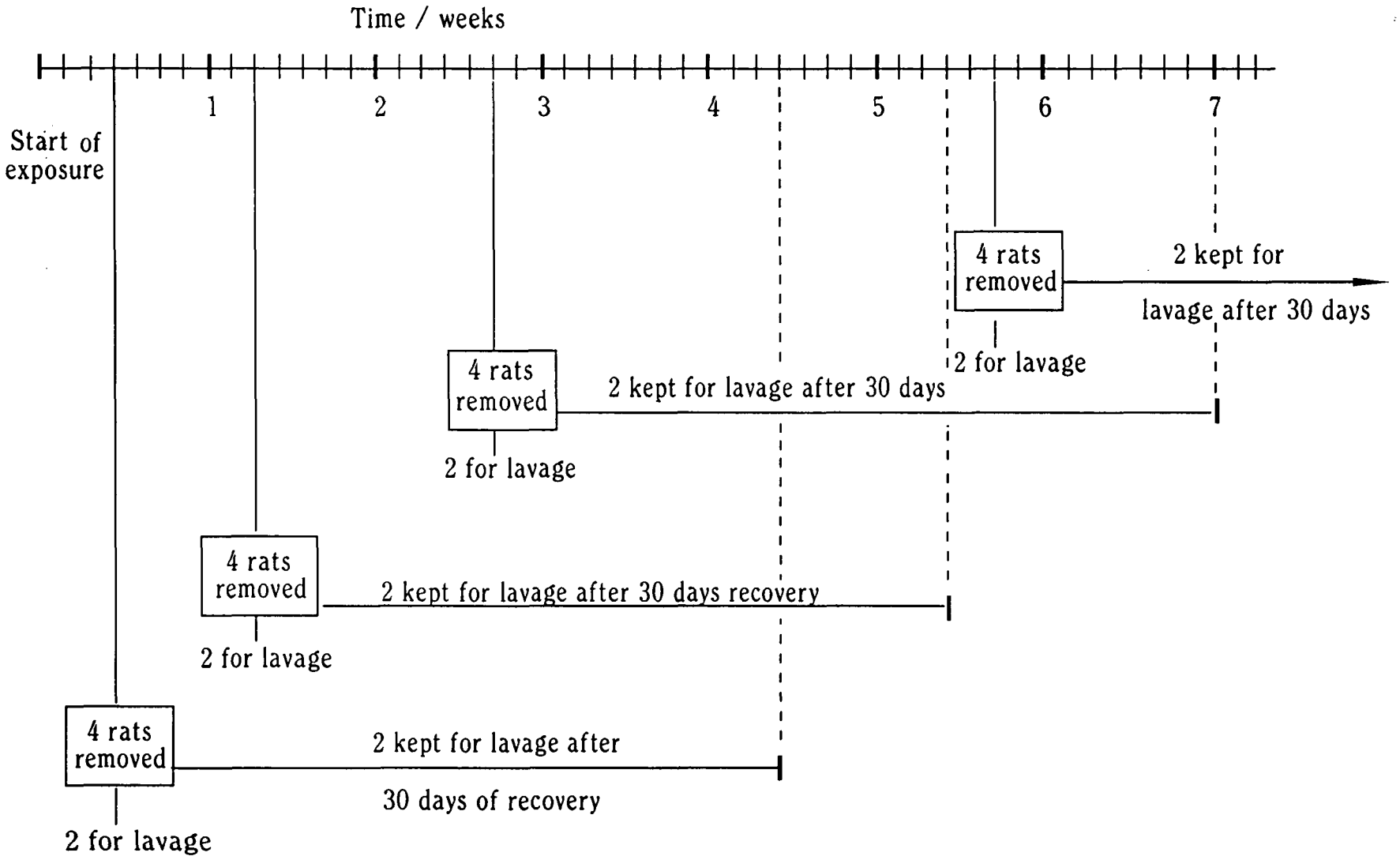
exposure to coalmine dust at 20 mg/m^3 for 7 hours per day, 5 days per week



Experiment terminated 28 months after commencement of exposure when the surviving rats were 31 months old.

Figure 2

Short term inhalation exposures to examine early pulmonary response exposure to coal mine dust at 20 mg/m^3 for 7 hours per day, 5 days per week.



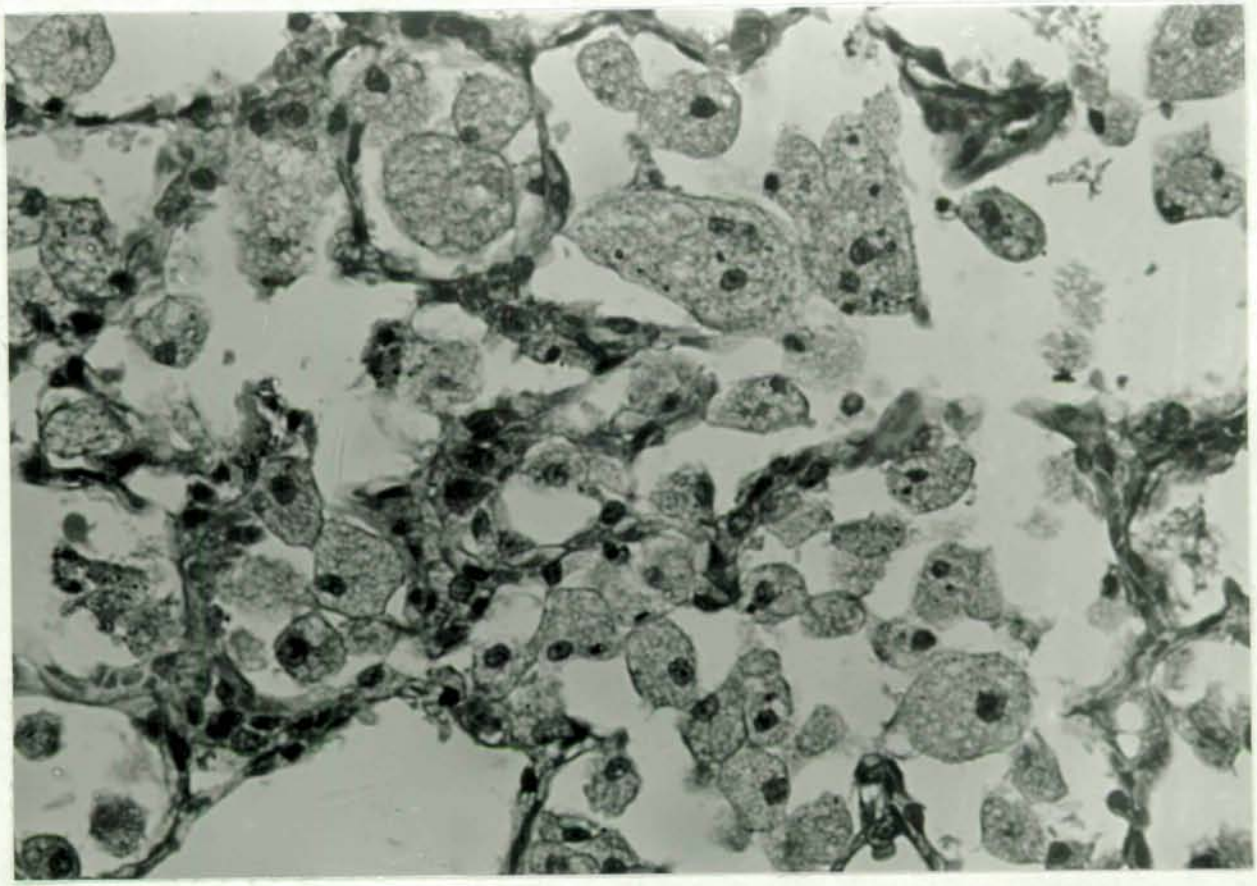


Figure 3 Pulmonary alveoli containing enlarged 'foamy' macrophages from the lung of a rat treated with dust from the Nottinghamshire colliery for 12 months.

Magnification x 450

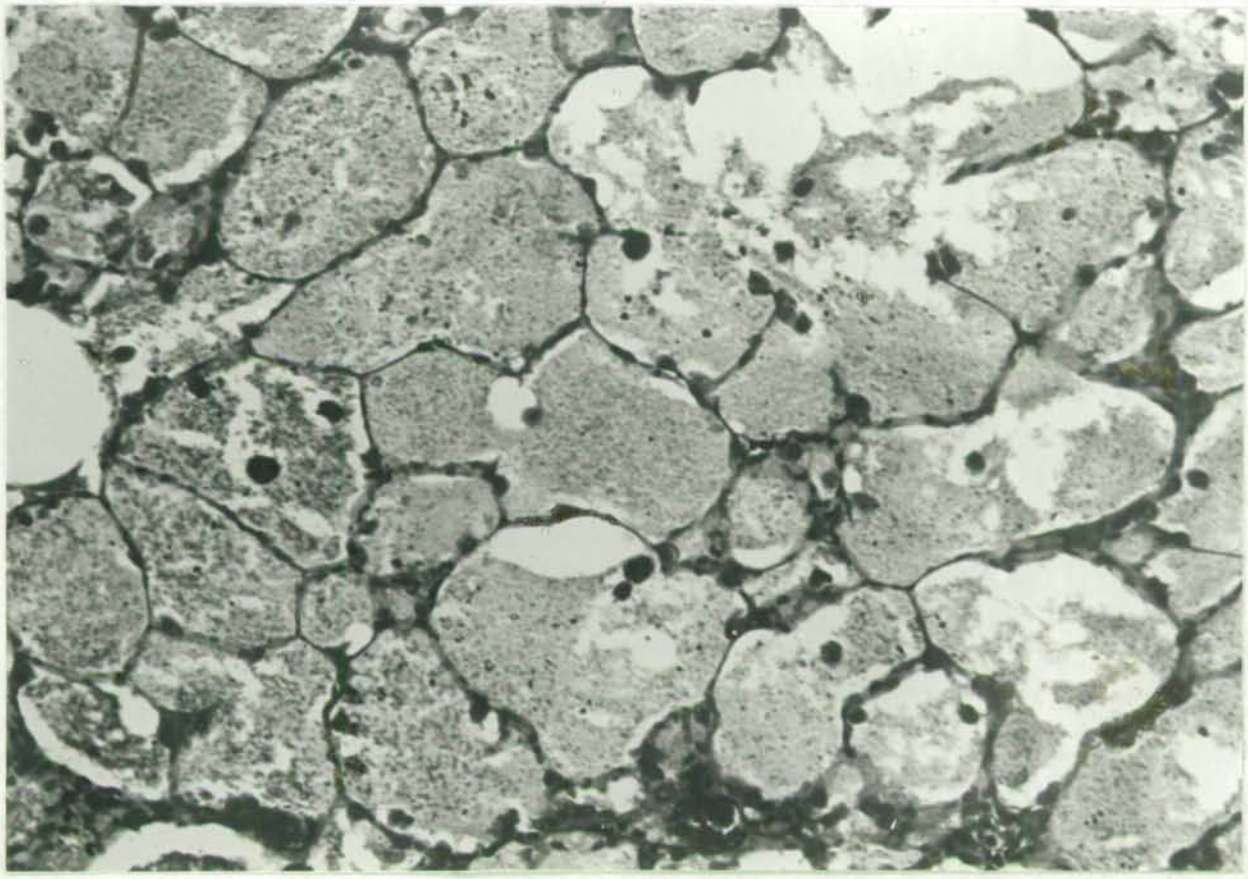


Figure 4 Pulmonary alveoli filled with lipoproteinaceous material in the lung of a rat treated with dust from the Nottinghamshire colliery for 12 months. Few cells are present in the alveolar spaces but some dense dust particles are visible embedded in the masses of lipoprotein.

Magnification x 200

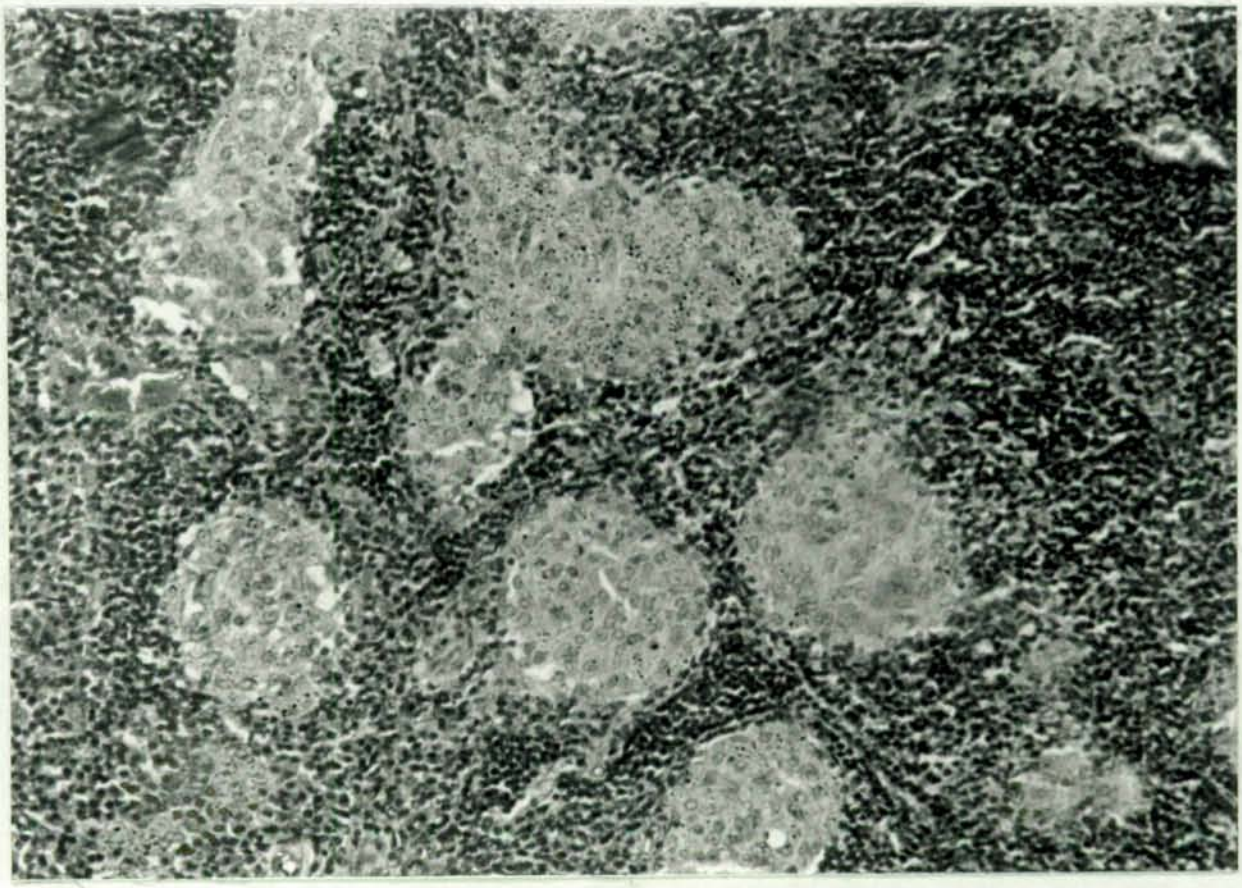


Figure 5 Section of a hilar lymph node from a rat treated for 12 months with dust from the Nottinghamshire colliery. Masses of pale cells of epitheloid type are present among the densely staining lymphocytes and these contain small amounts of mine dust particles.

Magnification x 250

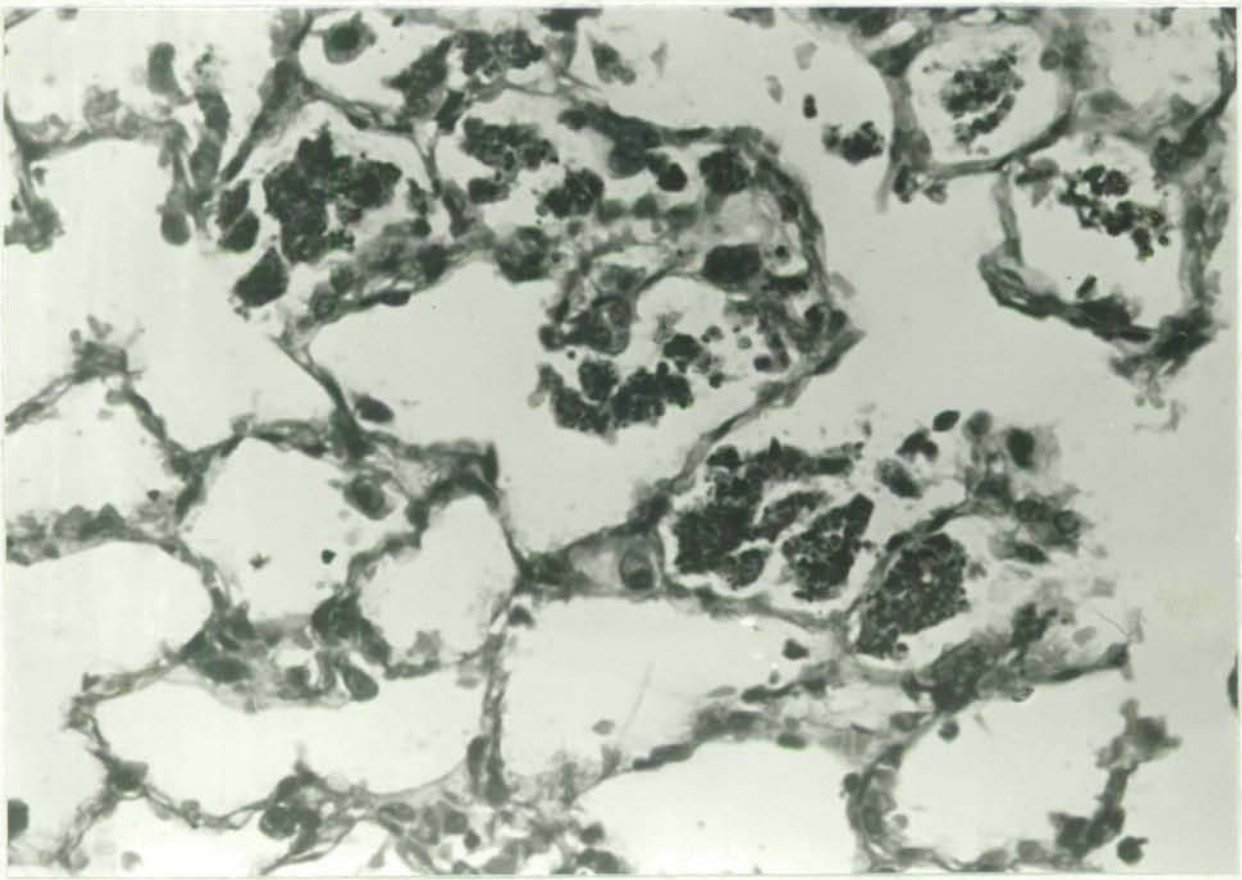
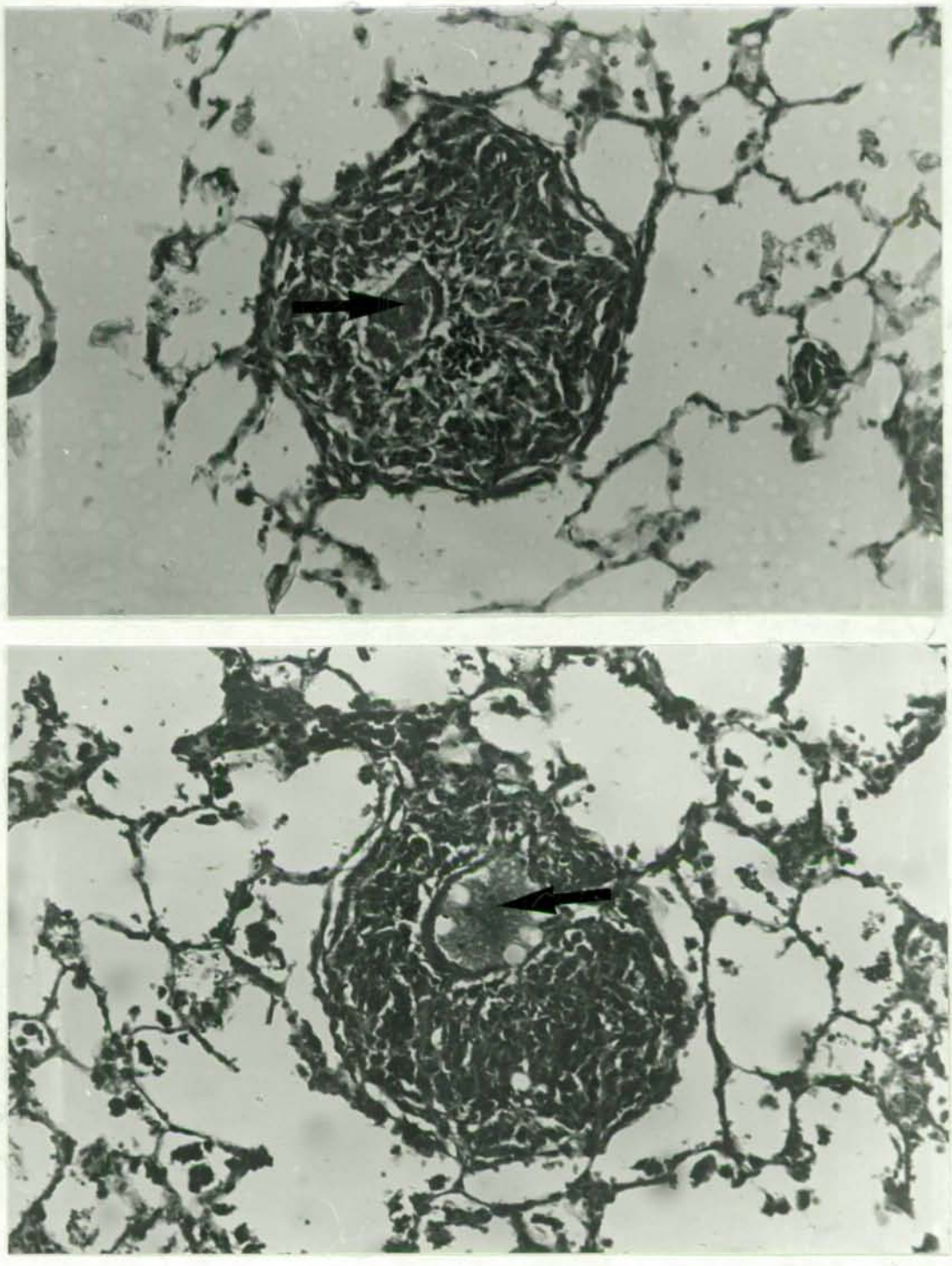


Figure 6 An area of pulmonary alveoli close to an alveolar duct from the lungs of a rat treated with dust from the Scottish colliery for 18 months. The alveoli closest to the duct contain aggregates of pulmonary macrophages each of which have phagocytosed many particles of mine dust.

Magnification x 400



Figures 7 & 8 Small nodules consisting of cells of epitheloid type and containing mine dust particles formed around small blood vessels (arrowed) in the lungs of a rat treated with dust from the Scottish colliery for 18 months followed by 10 months in dust free conditions.

Magnification x 200

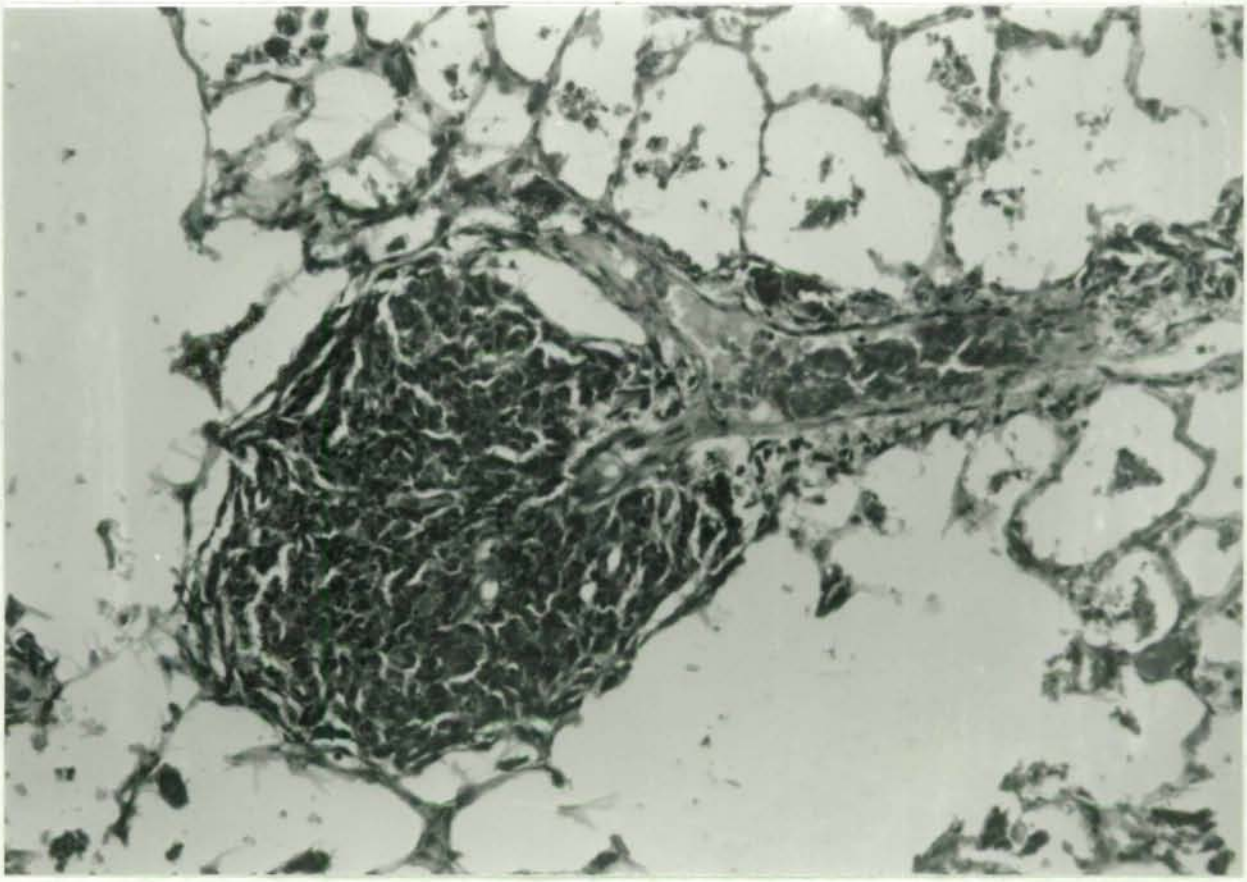


Figure 9 A small nodule of epitheloid cells, with dust, formed around one branch of a small blood vessel in the lungs of a rat treated with dust from the Scottish colliery for 18 months followed by 10 months in dust free conditions. The section is taken close to a bifurcation in the vessel and shows that only one of the two adjacent branches has become involved in nodule formation.

Magnification x 200

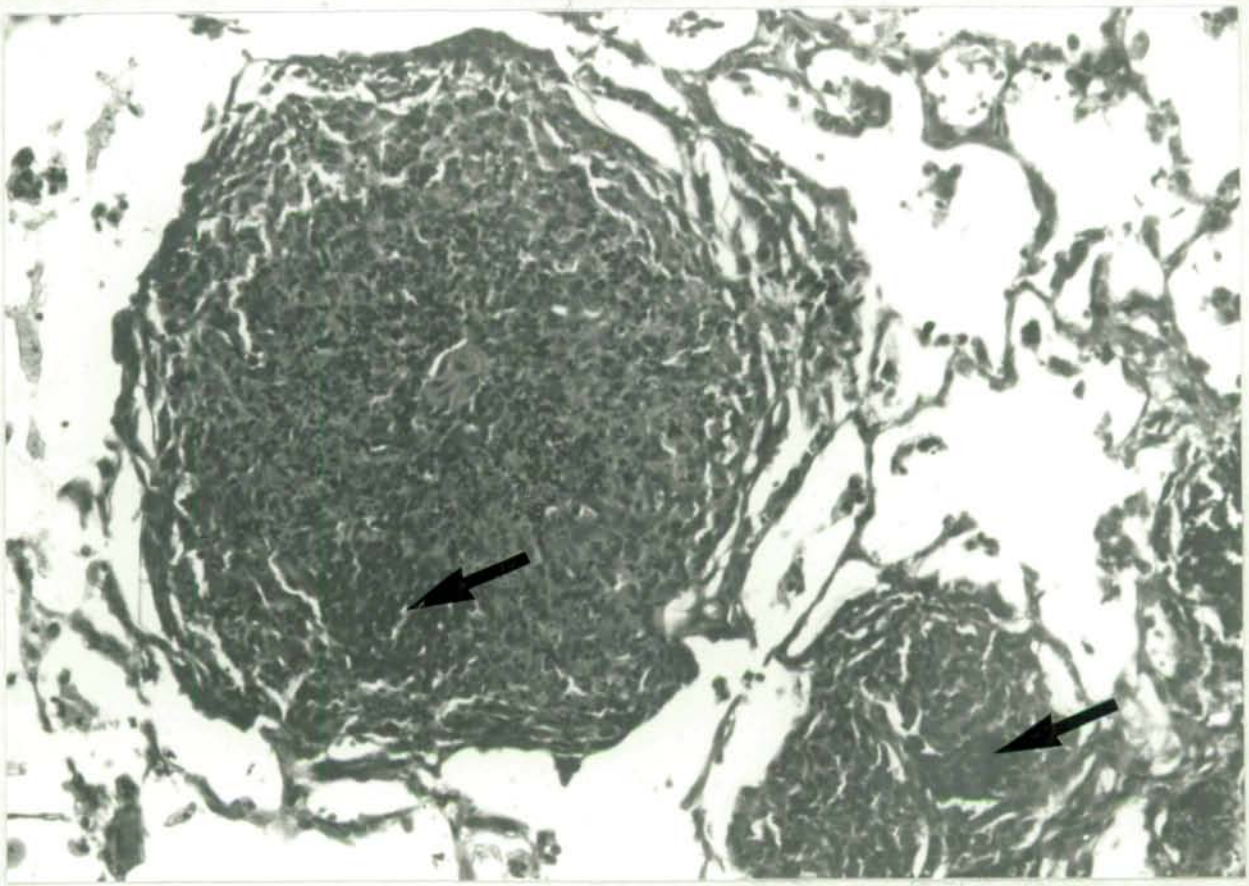


Figure 10 Perivascular nodules in the lungs of a rat treated with dust from the Scottish colliery for 18 months followed by 10 months in dust free conditions. The larger nodule is approximately 0.5mm in diameter. Both nodules consist mainly of cells of epitheloid type but in each case clusters of lymphocytes are present (arrowed).

Magnification x 200

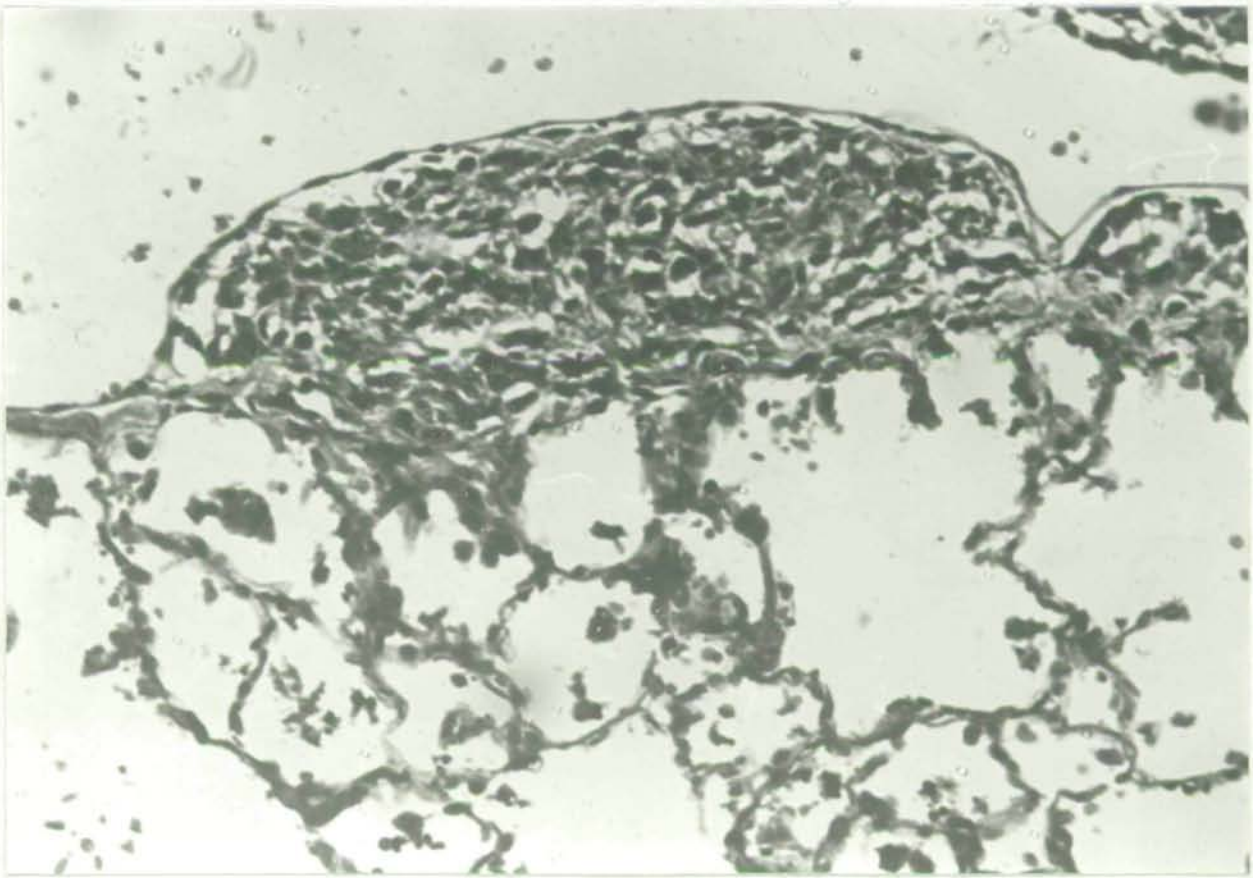


Figure 11 A granuloma formed on the visceral pleural surface of the lungs of a rat treated with dust from the Scottish colliery for 18 months followed by 10 months in dust free conditions. Although the overall structure is looser, the cellular elements of this lesion are similar to those of the pulmonary perivascular nodules and dust particles are present.

Magnification x 200

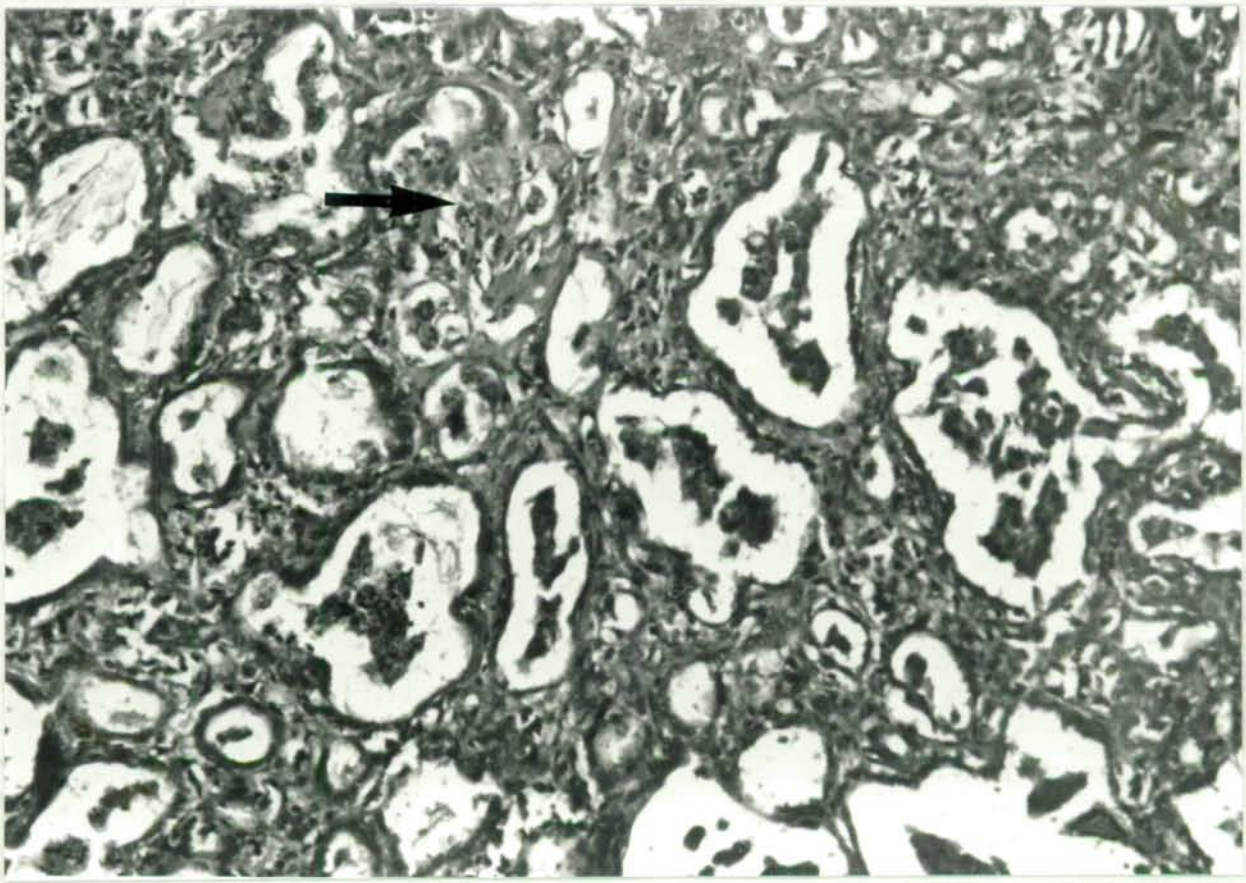


Figure 12 An area of lung tissue from the lung of a rat treated with dust from the Nottinghamshire colliery for 18 months followed by 10 months in dust free conditions. The alveoli are distorted and the alveolar walls greatly thickened by granulation tissue cells and connective tissue fibres. Both reticulin and collagen staining are present. The alveoli are lined with cuboidal cells and the alveolar spaces contain macrophages with numerous dust particles. In one instance (arrowed) an alveolus shows evidence of complete consolidation with early fibrosis.

Magnification x 250

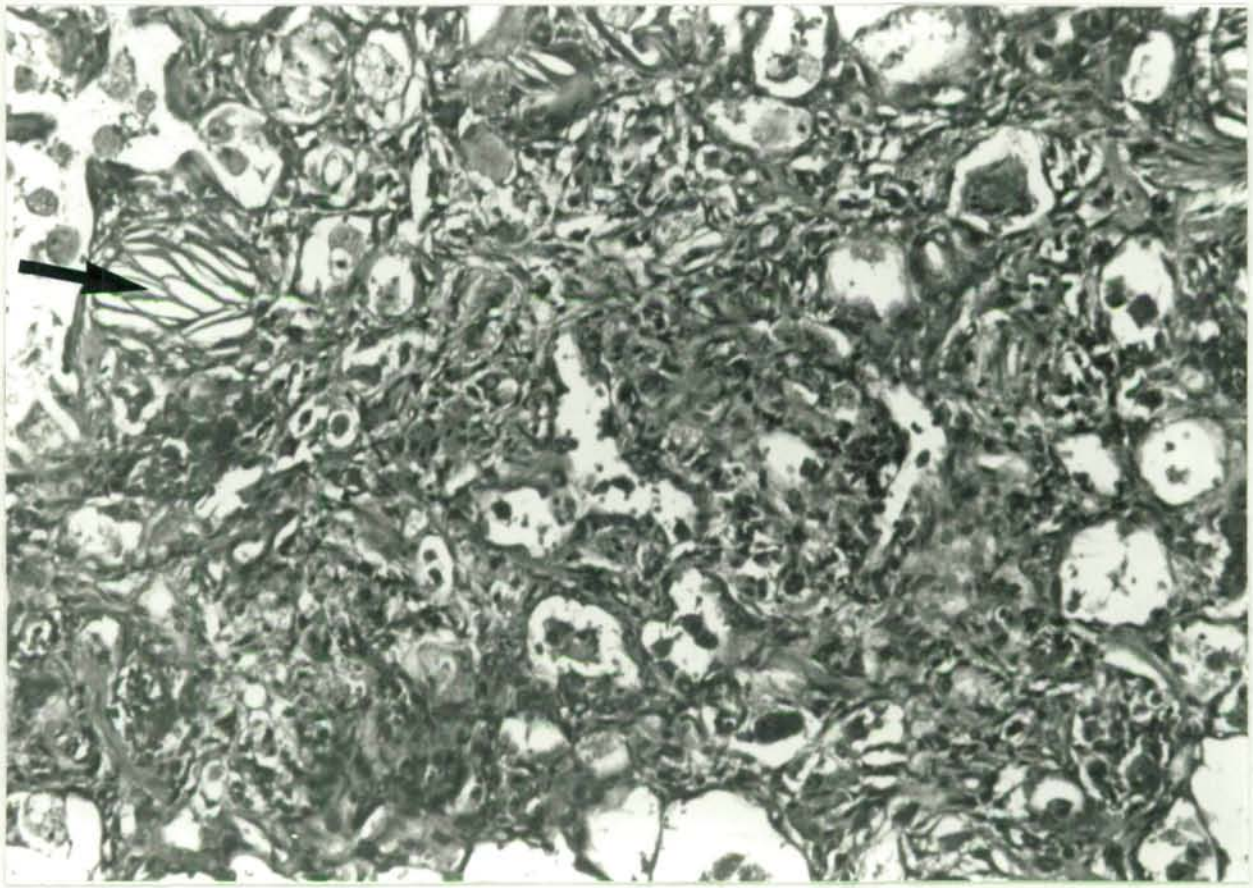


Figure 13 An area of lung tissue from the lung of a rat treated with dust from the Scottish colliery for 18 months followed by 10 months in dust free conditions. At this point the lung has become consolidated with fibrosing granulation tissue containing small alveolar remnants still lined by rounded epithelial cells. One area, probably representing a complete alveolus is filled with cholesterol crystals between which are compressed cells (arrowed).

Magnification x 250

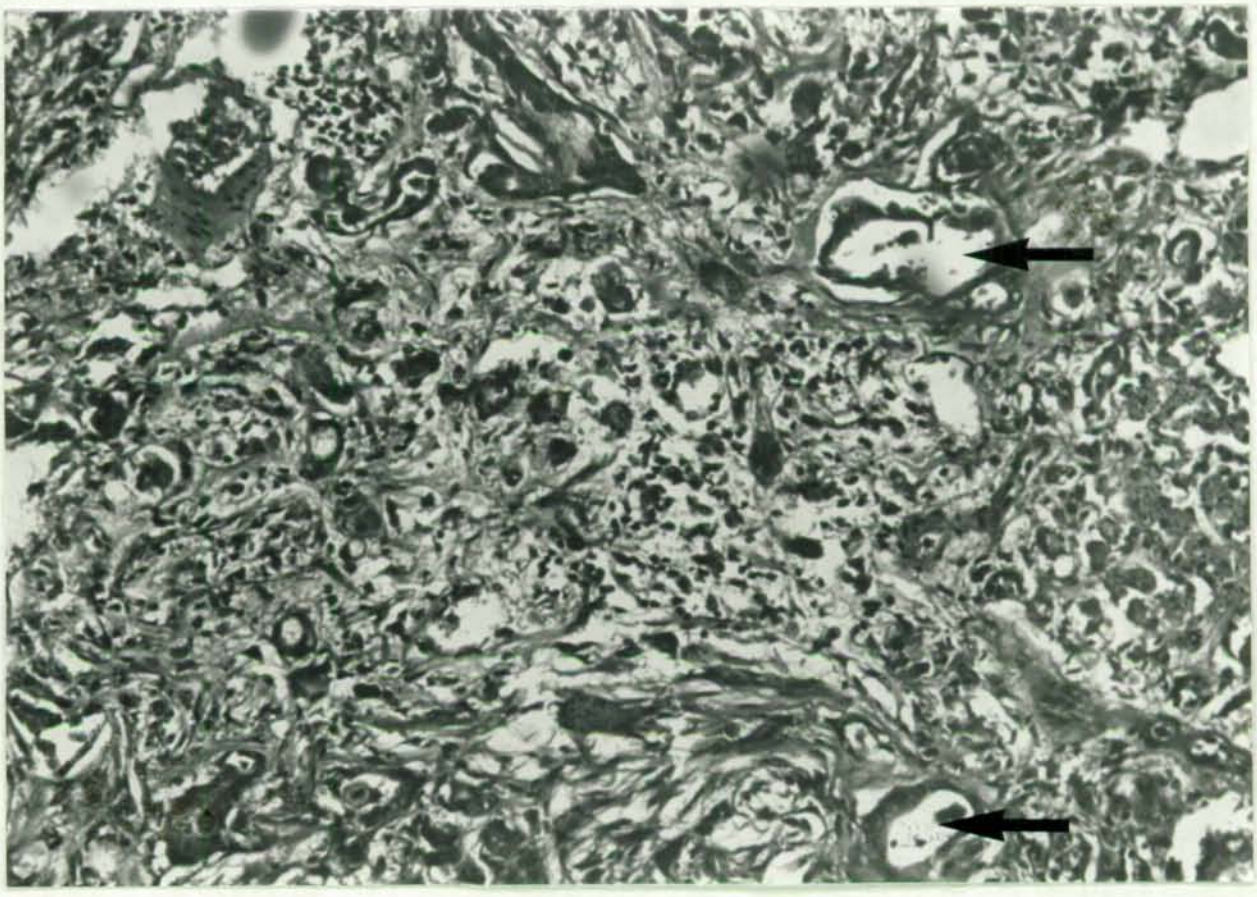


Figure 14 An area of lung tissue from the lung of a rat treated with dust from the Nottinghamshire colliery for 18 months followed by 10 months in dust free conditions. In this area complete consolidation has occurred although while the lower part of the photograph showed relatively advanced fibrosis, the central and upper areas consist of much younger granulation tissue. A few alveolar remnants remain, lined by rounded epithelial cells (arrowed).

Magnification x 250

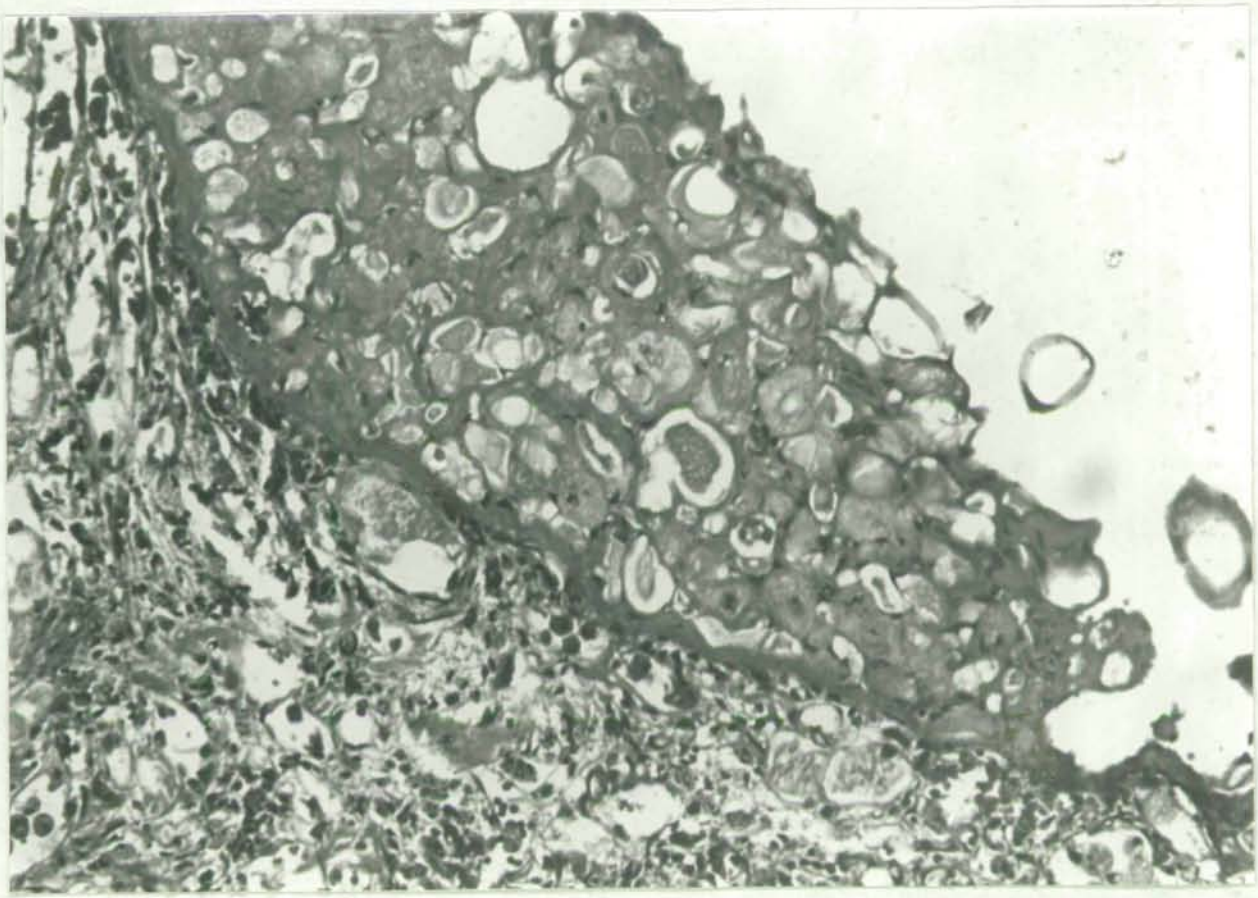


Figure 15 An area of vescicular fibrosis on the visceral pleural surface of a rat treated with the Scottish dust for 18 months followed by 10 months in dust free conditions. The lesion consists of loose fibrous tissue, staining positive for both reticulin and collagen, which contains numerous spaces lined by flattened cells of mesothelial type. No dust particles are present within this whole area.

Magnification x 250

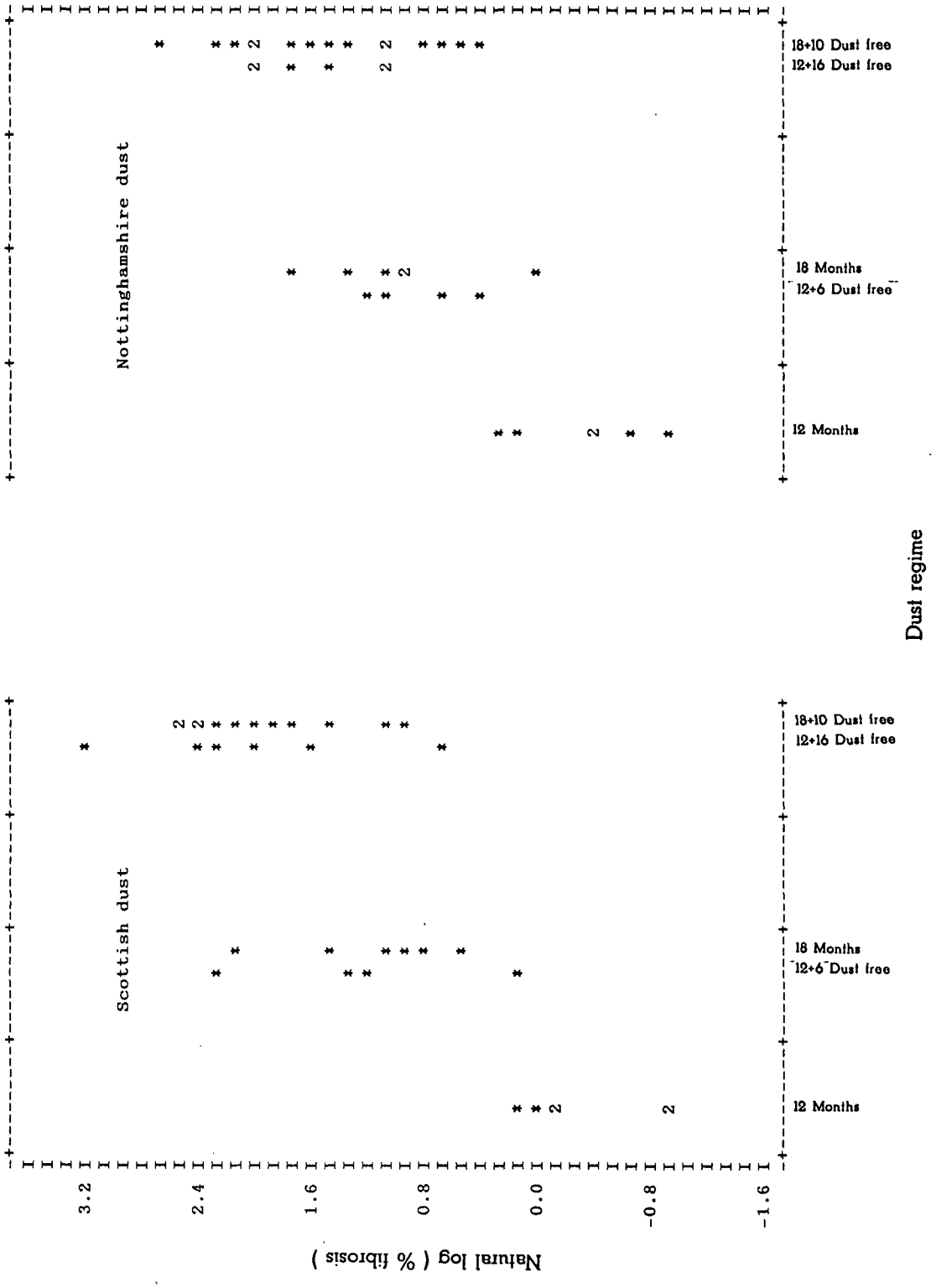


Figure 16 Variations in levels of pulmonary fibrosis in the lungs of rats treated for varying periods with coalmine dusts from Scotland and Nottinghamshire. For statistical comparison, the natural logs of the data are plotted.

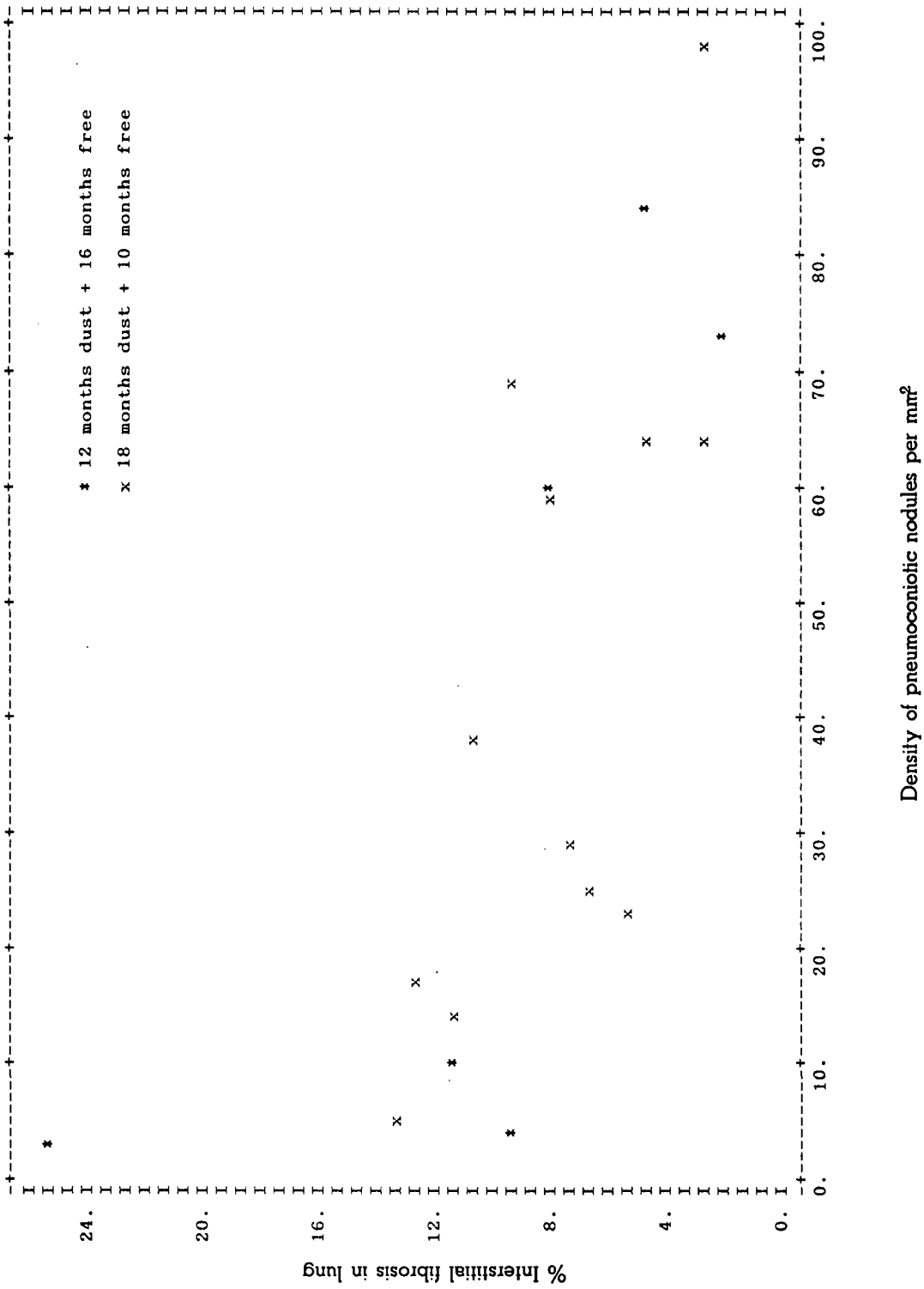


Figure 17 Comparisons of the numbers of pneumoconiotic nodules produced in the lungs of rats treated with the Scottish dust compared to the percentage of lung tissue involved with alveolar interstitial fibrosis.

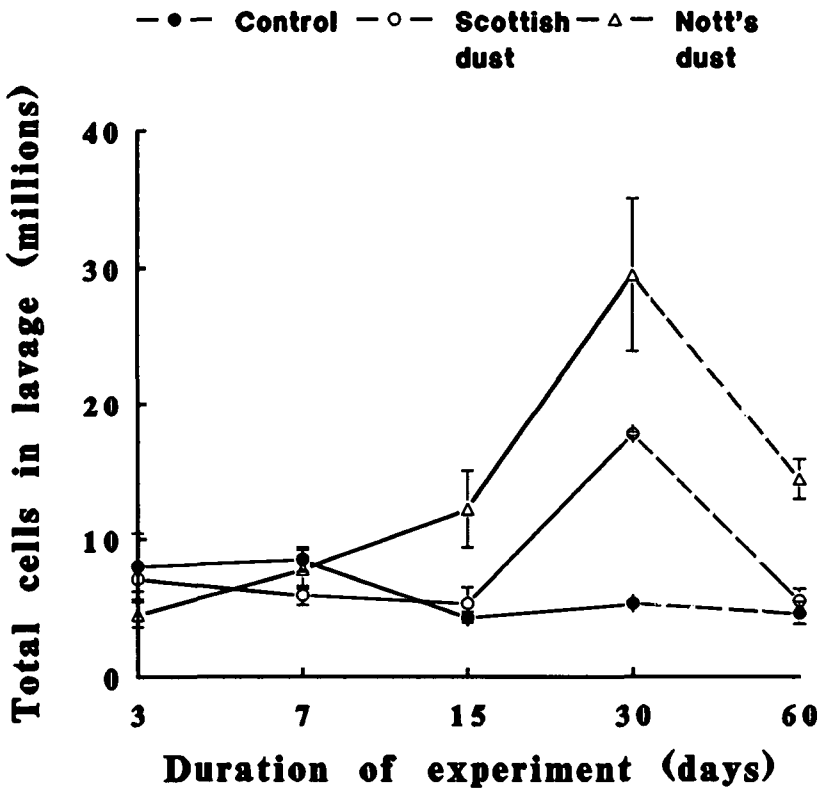


Figure 18 Numbers of pulmonary leukocytes lavaged from the lungs of rats exposed to two coalmine dusts. The animals were killed at varying timepoints during a 30 day inhalation period or after a 30 day inhalation period followed by 30 days recovery. Error bars indicate the standard error of the mean.

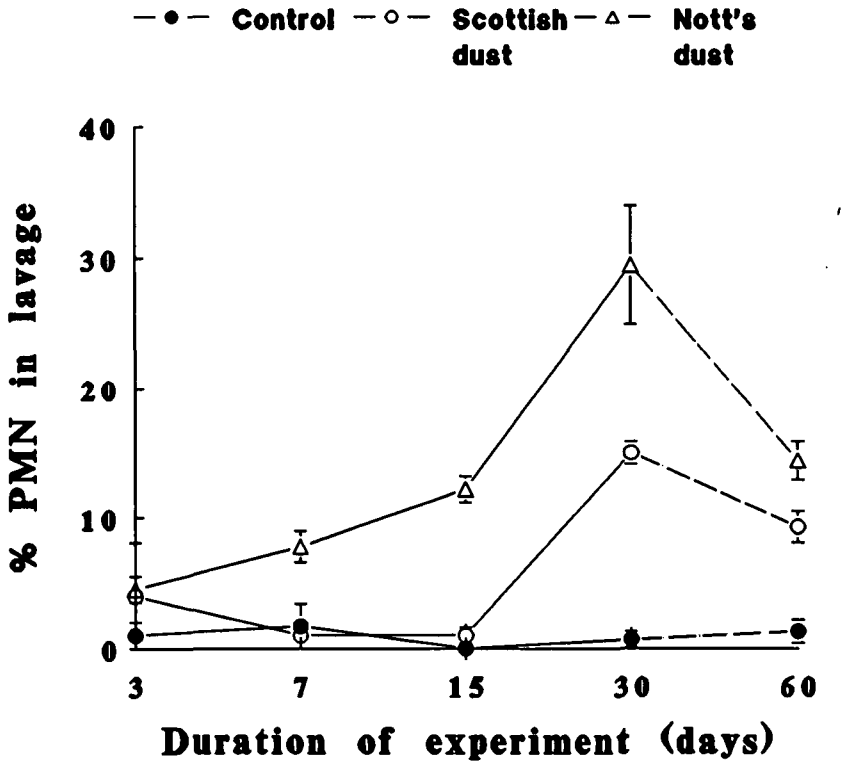


Figure 19 The percentage of neutrophil leukocytes lavaged from the lungs of rats exposed to two coalmine dusts. The animals were killed at varying timepoints during a 30 day inhalation period or after a 30 day inhalation period followed by 30 days recovery. Error bars indicate the standard error of the mean.

TABLE 1 Summary data from the mineralogical analysis of respirable dust clouds used in the experimental phase of this study. Ashing undertaken at 380°C

(a) Summary data from the Scottish dust

	N	MEAN	MEDIAN	STDEV	SEMEAN	MIN	MAX
% ash	21	75.55	76.50	3.5	0.77	69.40	80.70
ASH							
% quartz	21	24.30	24.40	1.84	0.40	19.00	26.50
DUST							
% quartz	30	18.41	18.50	1.58	0.29	14.20	20.80

(b) Summary data from the Nottinghamshire dust

	N	MEAN	MEDIAN	STDEV	SEMEAN	MIN	MAX
% ash	18	86.31	86.95	3.27	0.77	76.30	90.70
ASH							
% quartz	18	18.21	18.20	0.73	0.17	16.90	20.20
DUST							
% quartz	27	15.72	15.60	0.79	0.15	14.30	18.30

TABLE 2 Quartz and ash analyses undertaken to check on the uniformity of resirable dust clouds generated with dust from the Scottish and Nottinghamshire collieries during the experimental inhalation period.

	SCOTTISH DUST				NOTTINGHAMSHIRE DUST		
SAMPLE NUMBER	ASH %	QUARTZ % IN ASH	QUARTZ % IN DUST	SAMPLE NUMBER	ASH %	QUARTZ % IN ASH	QUARTZ % IN DUST
1	75.2	19.0	14.3	1	90.7	20.2	18.3
2	77.9	26.0	20.3	2			16.4
3	77.3	26.2	20.3	3			14.8
4	77.1	25.5	19.7	4			15.6
5			18.9	5			15.2
6			18.1	6	NOT DETERMINED		16.6
7			18.6	7			16.0
8			18.2	8			15.4
9	NOT DETERMINED		20.8	9			15.9
10			18.3	10			15.7
11			17.9	11	84.6	18.4	15.6
12			18.6	12	87.1	18.1	15.8
13			17.3	13	89.3	17.9	16.0
14	79.3	23.5	18.6	14	89.3	18.0	16.1
15	79.4	24.8	19.7	15	87.2	16.9	14.7
16	78.1	25.9	20.2	16	83.8	18.4	15.4
17	79.1	24.9	19.7	17	87.9	18.9	16.6
18	77.6	24.2	18.8	18	86.0	17.8	15.3
19	77.1	25.6	19.7	19	84.1	18.1	15.2
20	71.6	25.7	18.4	20	84.2	18.5	15.6
21	70.2	25.1	17.6	21	76.3	18.8	14.3
22	69.4	24.3	16.7	22	84.8	18.3	15.5
23	75.1	26.5	19.9	23	86.8	18.6	16.1
24	69.5	20.4	14.2	24	88.4	17.2	15.2
25	69.7	23.7	16.5	25	85.5	17.3	14.8
26	76.5	23.0	17.6	26	87.9	17.9	15.7
27	76.0	24.1	18.3	27	89.7	18.6	16.7
28	75.2	24.4	18.3				
29	74.6	23.1	17.2				
30	80.7	24.4	19.7				

TABLE 3 Mean levels of alveolar interstitial fibrosis in the lungs of rats treated with dusts from collieries in Nottinghamshire and Scotland. Fibrosis is expressed as a percentage of lung tissue area.

Dusting regime	Animal numbers	Scottish dust	Nottinghamshire dust
12 month dusting	6	0.64	0.72
18 months dusting	6	3.61	3.15
12 months dusting 6 months dust free	4	4.55	2.47
18 months dusting 10 months dust free	12 Scottish dust 15 Nottinghamshire dust	7.95	5.11
12 months dusting 16 months dust free	6	10.22	5.15

TABLE 4 Areas of alveolar interstitial fibrosis in the lungs of individual animals treated with dust from coalmines in Nottinghamshire and Scotland. Fibrosis is expressed as a percentage of lung tissue area.

Dusting regime	Animal numbers	Scottish dust	Nottinghamshire dust
	1	0.87	0
	2	1.13	0.52
	3	0	0.64
12 months dusting	4	1.01	0.71
	5	0.84	1.30
	6	0	1.12
		Mean 0.64	0.72
	1	1.74	2.65
	2	2.17	0.96
	3	8.11	3.68
18 months dusting	4	2.39	2.66
	5	4.21	2.95
	6	3.05	5.97
		Mean 3.61	3.15
	1	9.86	3.50
12 months dusting	2	3.46	3.02
6 months clean air	3	1.20	1.48
	4	3.68	1.88
		Mean 4.55	2.47
18 months dusting	1	8.10	2.29
10 months dust free	2	11.46	7.00
	3	13.43	3.77
	4	6.69	4.22
	5	4.50	4.78
	6	2.81	13.47
	7	7.61	9.06
	8	2.64	7.43
	9	10.71	5.54
	10	9.44	2.03
	11	12.45	1.75
	12	5.57	3.03
	13		1.48
	14		2.82
	15		7.97
		Mean 7.95	5.11
12 months dusting	1	11.61	2.92
16 months clean air	2	25.55	7.22
	3	1.94	2.83
	4	4.92	5.99
	5	9.55	7.62
		7.79	4.32
		Mean 10.22	5.15

TABLE 5 Numbers of pneumoconiotic nodules developing in the lungs of rats treated with dust from the Scottish colliery compared to levels of alveolar interstitial fibrosis.

Figures for nodules are numbers per square centimetre of tissue. Interstitial fibrosis figures are percentages of the total area of lung tissue

Dusting regime	Animal number	Numbers of pneumoconiotic nodules	Area of interstitial fibrosis
Group A	1	9.60	11.61
12 months			
dusting	2	2.57	25.55
16 months	3	72.99	1.94
dust free	4	84.05	4.92
	5	4.29	9.55
	6	60.42	7.79
		Mean 38.98	Mean 10.23
Group B	1	59.50	8.10
18 months	2	14.37	11.46
dusting	3	4.97	13.43
10 months	4	25.40	6.69
dust free	5	64.38	4.50
	6	63.82	2.81
	7	29.30	7.61
	8	98.19	2.64
	9	38.07	10.71
	10	69.30	9.44
	11	17.48	12.45
	12	22.69	5.57
		Mean 42.29	Mean 7.95

TABLE 6 Summary of interstitial fibrosis data. Tabulated for each kill group are number of data values = animals, and mean and variance of natural log of % fibrosis.

		SCOTTISH DUST			NOTTINGHAMSHIRE DUST		
Regime dust + free	Age at kill (m)	no	means	vars	no	means	vars
12 + 00	12	6	-0.34	0.2136	6	-0.33	0.2020
12 + 06	18	4	1.25	0.7404	4	0.85	0.1616
18 + 00	18	6	1.14	0.3093	6	1.01	0.3601
Combined	18	10	1.19	0.4220	10	0.95	0.2615
12 + 16	28	6	2.04	0.7509	6	1.56	0.1933
18 + 10	28	12	1.95	0.3028	15	1.43	0.4334
Combined	28	18	1.98	0.4187	21	1.47	0.3552

TABLE 7 Fitted values from ANOVA of interstitial fibrosis data with main effects of age and mine

	SCOTTISH		NOTTINGHAMSHIRE	
	mean	s.e.	mean	s.e.
age				
12	-0.16	0.183	-0.51	0.183
18	1.24	0.149	0.89	0.149
28	1.89	0.120	1.55	0.114

TABLE 8 Mass weights of mine dusts and their mineral components extracted from rat lungs at the listed timepoints. Figures are in milligrammes

(a) Dust from the Scottish colliery

Inhalation period plus follow-up (months)	No. of rats	Total dust	Coal	Ash	Quartz	Kaolin	Illite
12 + 0	6	14.3	2.9	11.4	3.0	4.9	2.6
12 + 6	4	13.6	2.1	11.5	2.9	5.3	1.7
12 + 16	5	13.8	4.3	9.4	2.1	4.2	1.1
18 + 0	6	22.3	3.2	19.1	5.6	8.7	4.3
18 + 10	6	19.2	4.2	14.9	3.9	7.1	2.0

(b) Dust from the Nottinghamshire colliery

Inhalation period plus follow-up (months)	No. of rats	Total dust	Coal	Ash	Quartz	Kaolin	Illite
12 + 0	6	20.7	2.2	18.5	4.7	4.1	8.0
12 + 6	4	22.1	3.4	18.7	4.7	4.3	6.3
12 + 16	5	18.7	4.9	13.8	3.3	3.0	4.4
18 + 0	6	31.8	5.8	25.9	5.8	5.7	8.7
18 + 10	6	28.3	6.2	22.1	5.5	4.9	8.8

TABLE 9 Percentage composition of original mine dusts and dusts extracted from rat lungs at the listed timepoints

(a) Dust from the Scottish colliery

Inhalation period plus follow-up (months)	No. of rats	Coal	Ash	Quartz	Kaolin	Illite
Original dust	-	24.4	75.5	18.4	25.5	17.0
12 + 0	6	20.4	79.5	21.4	34.6	18.1
12 + 6	4	15.3	84.6	21.6	39.3	12.4
12 + 16	5	31.5	68.5	15.5	30.3	8.0
18 + 0	6	14.3	85.7	25.1	39.1	19.2
18 + 10	6	22.2	77.7	20.2	37.2	10.4

(b) Dust from the Nottinghamshire colliery

Inhalation period plus follow-up (months)	No. of rats	Coal	Ash	Quartz	Kaolin	Illite
Original dust	-	13.6	86.3	15.7	18.6	32.3
12 + 0	6	10.7	89.2	22.6	19.9	38.7
12 + 6	4	15.5	84.4	21.2	19.4	28.6
12 + 16	5	26.4	73.5	17.7	16.1	23.2
18 + 0	6	18.4	81.5	18.4	18.0	27.3
18 + 10	6	21.8	78.1	19.6	17.5	31.0

Table 10 Total numbers of leukocytes in the bronchoalveolar lavage of control rats and those exposed to 10mg/m^3 of airborne Scottish or Nottinghamshire coalmine dusts. Results are the mean (SEM) of 3 rats per group at each time.

Duration of exposure (days)	Control	Scottish	Nottinghamshire
3	6.4 (1.3)	7.1 (1.5)	4.5 (1.0)
3 + Rec	7.1 (0.4)	6.3 (0.4)	7.3 (0.7)
7	6.9 (0.9)	5.9 (0.7)	7.8 (1.2)
7 + Rec	7.7 (1.0)	7.5 (2.0)	7.9 (0.6)
15	4.3 (0.4)	5.3 (0.2)	12.2 (1.0)**
15 + Rec	6.9 (1.6)	9.7 (0.7)	14.9 (2.1)**
30	5.3 (0.1)	17.7 (2.4)***	29.5 (4.5)***
30 + Rec	4.6 (0.8)	5.5 (0.9)	14.4 (1.5)***

Recovery period 30 days in all cases
Significantly greater than control:-
** $p<0.001$
*** $p<0.005$

Table 11 Percentage of neutrophils in the bronchoalveolar lavage of control rats and those exposed to 10mg/m^3 of airborne Scottish or Nottinghamshire coalmine dusts. Results are the mean (SEM) of 3 rats per group at each timepoint.

Duration of exposure (days)	Control	Scottish	Nottinghamshire
3	0.5 (0.5)	4.0 (4.1)	0 (0)
3 + Rec	0.7 (0.8)	0 (0)	0.5 (0.4)
7	0.8 (0.5)	1.0 (1.0)	13.7 (5.9)*
7 + Rec	0.5 (0.3)	1.0 (0.6)	1.3 (0.7)
15	0 (0)	1.0 (0.6)	29.0 (5.0)**
15 + Rec	0 (0)	0 (0)	17.3 (1.7)***
30	0.7 (0.6)	15 (0.9)***	41.0 (5.8)**
30 + Rec	1.3 (0.9)	9.3 (1.2)***	27.3 (2.7)***

Recovery period 30 days in all cases
Significantly greater than control

* $p<0.05$
** $p<0.0025$
*** $p<0.001$

TABLE 12 Levels of fibronectin proteolysis in leukocyte populations from the lungs of rats treated with samples of mine dust from Scotland and Nottinghamshire.

	SCOTTISH DUST	NOTTINGHAMSHIRE DUST
3	98	101
3 + Rec	102	79
7	98	126
7 + Rec	105	108
15	148*	148**
15 + Rec	97	116*
30	104	108
30 + Rec	104	116

Recovery period 30 days in all cases
* dust greater than control, $p<0.025$
** dust greater than control, $p<0.001$

Half-life effects of the ^{125}Iodine used to radiolabel the fibronectin in this assay confound the direct comparison between groups of absolute counts. The data are therefore expressed as fibronectin proteolysis by dust-elicited leukocytes as a percentage of control leukocyte proteolytic activity.

TABLE 13 Chemotaxis in leukocyte populations from the lungs of rats treated with samples of mine dust from Scotland and Nottinghamshire.

EXPOSURE	CONTROL		SCOTTISH DUST		CONTROL		NOTTINGHAMSHIRE DUST	
	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
3 Day	36.6	4.9	33.2	4.8	23.3	2.9	27.0	3.4
3 Day + Rec†	49.7	3.5	49.8	5.5	37.0	3.3	43.2	4.7
7 Day	56.7	5.3	30.3	3.4**	ND		ND	
7 Day + Rec†	44.8	5.0	48.7	4.8**	66.9	7.8	40.6	6.3*
15 Day	59.1	3.4	10.3	1.6***	59.1	3.4	3.6	0.8***
15 Day + Rec†	21.7	3.2	19.1	4.3	21.7	3.2	5.4	1.7**
30 Day	34.5	8.1	17.9	2.4*	34.5	8.1	7.0	1.2**
30 Day + Rec*	26.5	3.9	17.5	2.0	26.5	3.9	3.7	0.9***

† Recovery period 30 days in all cases

* dust less than control $p<.05$
** dust less than control $p<.01$
*** dust less than control $p<.005$

APPENDIX

The proportions of free quartz surface areas in the two coalmine dusts were compared using thermoluminescence and radioluminescence measurements by Dr W Kriegseis of the University of Giessen in Germany.

The basic method is to sediment about 10mg of dust on to a sample holder from a suspension in a 200:1 methanol-water mixture. The samples are heated in vacuo to get rid of loosely bound organic layers and then cooled to 77°K. The samples were irradiated by x-rays (30kV, 11As kg^{-1}) through a beryllium window at this temperature and then slowly heated. Light emitted by the sample during heating between 155K and at 250K was measured using a photomultiplier. Light emitted at 160K during heating of a sample after previous irradiation at 77K is referred to as the thermoluminescence. Light emitted at 155K and at 250K during heating up and during constant dose irradiation by x-rays is referred to as the radioluminescence.

The following notations are used and the following relationships (neglecting some proportionality factors) are assumed:

FQS = Free quartz surface
TL = Thermoluminescence intensity at 160 K
RL_{155}, RL_{250} = Radioluminescence intensity at 155 K and 250 K, respectively
dRL = Difference between RL_{155} and RL_{250}
PQ = Quartz content of dust in %
SP = Surface purity of the quartz,
Completely pure quartz surfaces SP = 1,
Completely coated quartz surfaces SP = 0

$$\text{Then } FQS = PQ.SP = C.TL \qquad (1)$$

where c is constant which takes account of attenuation of the emitted thermoluminescence caused by absorption and light scattering in the sample. This sample constant depends on dust composition and grain size among other things and can differ strongly from sample to sample.

The effect of attenuation can be corrected by measurement of radioluminescence at 170K. Quartz produces a strong radioluminescence emission below 170K which can be considered as a bulk effect (as opposed to a surface effect). At temperatures below 170K the RL intensity is much lower. Other components of coalmine dust also contribute weakly to radioluminescence but do not show the decrease at 170K characteristic of quartz. For this reason the difference dRL between the radioluminescence intensity at 155K and 250K can be assumed to be proportional to the bulk quartz content. Also, since the TL emission at 160K and the RL emission below 170K are only separated from each other by 80nm (wavelength) the light attenuation factors in the constant c will be practically the same.

The following equation is then valid:

$$dRL \times c = PQ \qquad (2)$$

in other words the attenuation factor c can be assessed by dividing the percentage quartz content by the radioluminescence difference ($RL_{155} - RL_{250}$).

$$c = \frac{PQ}{dRL} \qquad (3)$$

Then equation 1 becomes :

$$PQ\ SP = PQ \times TL/dRL$$

i.e.

$$SP = TL/dRL \qquad (4)$$

In addition, the measured thermoluminescence intensity at 160K has to be corrected by the subtraction of background emission at this temperature before it can be used in equation (4).

The results of the TL and RL measurements, and the TL/dRL ratios (using background corrected TL) are shown in the following table and in figures A1 and A2.

SAMPLE	RL		dRL	TL		TL/dRL
	155 K	250 K		uncorr	corr	
Nottinghamshire	19890	6900	12990	7573	3700	0.29
Scotland	16667	7041	9626	18281	14200	1.48

These figures show that the free quartz surface area in the Scottish dust is much larger than in the dust from Nottinghamshire by a factor of about 5. No mineral composition data or particle size data were available to Dr Kriegseis at the time of measurement and the 'free quartz surface' could only be determined in a simplified manner and not the normalised free quartz surface. However, since the dusts are reasonably similar in compositions the free quartz surfaces and the normalised free quartz surfaces will differ from each other by the same factor for both samples.

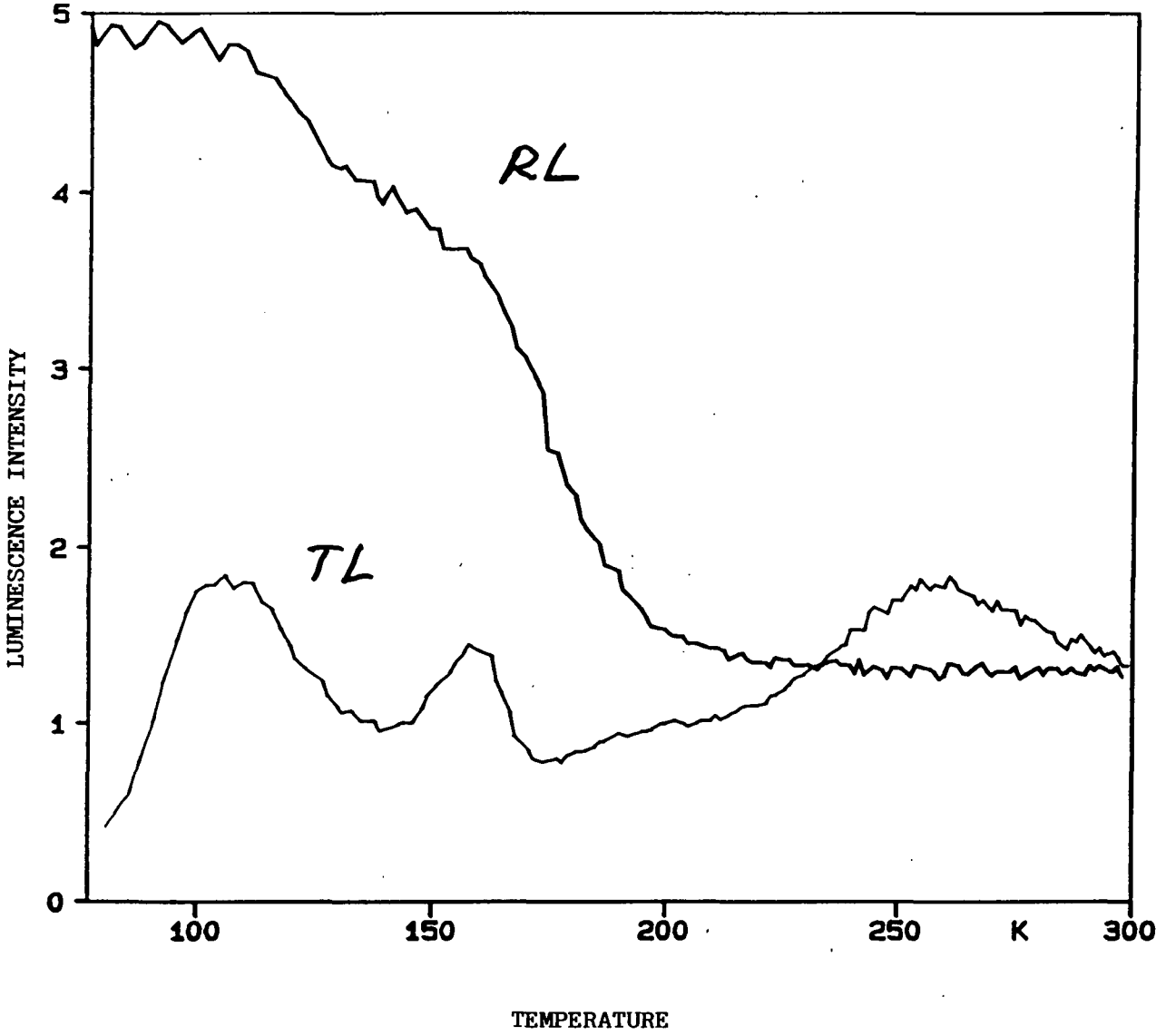


Figure A1 Thermoluminescence (TL) and radioluminescence (RL) intensities produced from the Nottinghamshire dust at varying temperatures

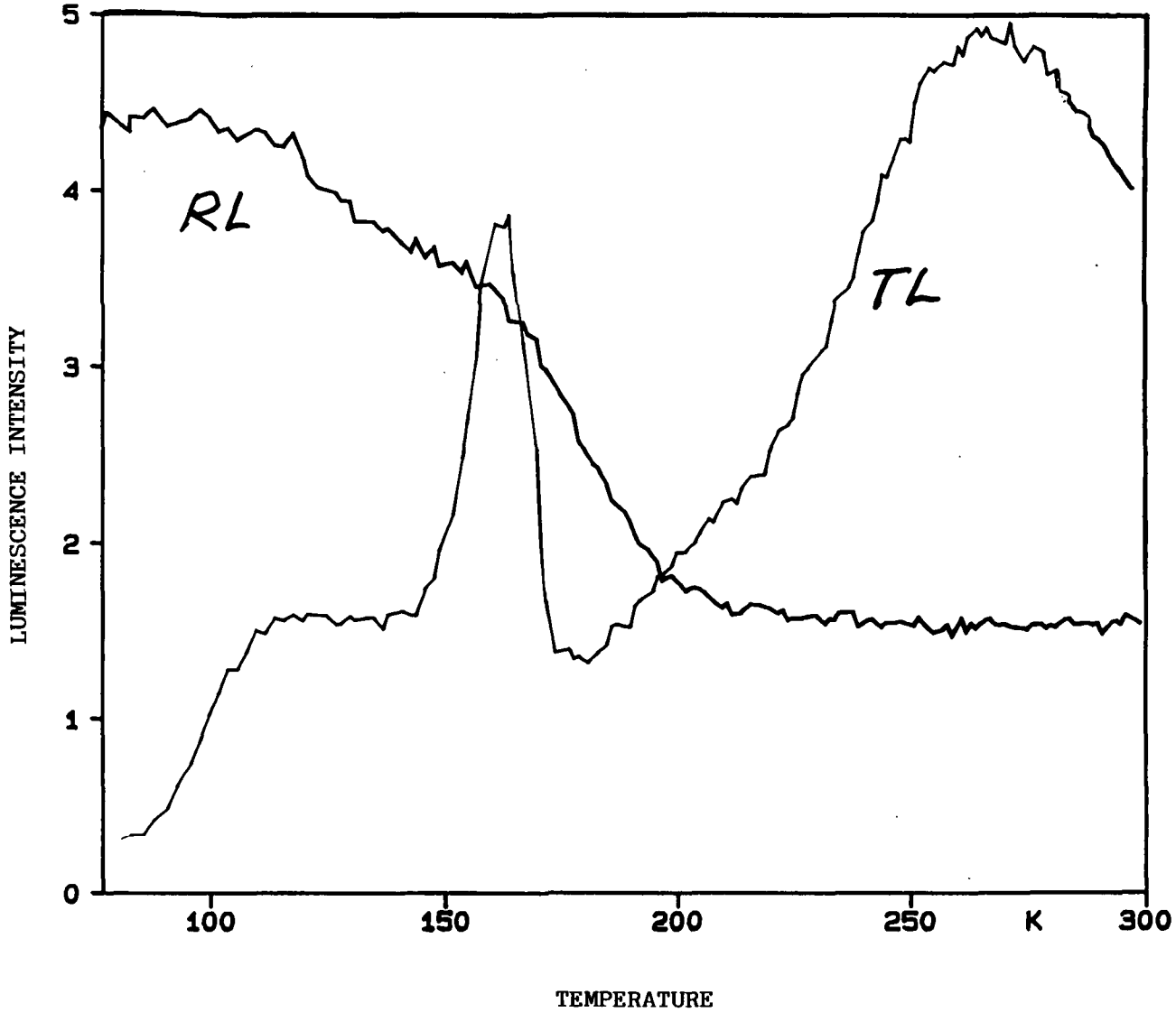


Figure A2 Thermoluminescence (TL) and radioluminescence (RL) intensities produced from the Scottish dust at varying temperatures

HEAD OFFICE:

Research Avenue North,
Riccarton,
Edinburgh, EH14 4AP,
United Kingdom
Telephone: +44 (0)870 850 5131
Facsimile: +44 (0)870 850 5132

Tapton Park Innovation Centre,
Brimington Road, Tapton,
Chesterfield, Derbyshire, S41 0TZ,
United Kingdom
Telephone: +44 (0)1246 557866
Facsimile: +44 (0)1246 551212

Research House Business Centre,
Fraser Road,
Perivale, Middlesex, UB6 7AQ,
United Kingdom
Telephone: +44 (0)208 537 3491/2
Facsimile: +44 (0)208 537 3493

Brookside Business Park,
Cold Meece,
Stone, Staffs, ST15 0RZ,
United Kingdom
Telephone: +44 (0)1785 764810
Facsimile: +44 (0)1785 764811

Email: iom@iom-world.org