



PRIVY COUNCIL

**MEDICAL RESEARCH COUNCIL**

SPECIAL REPORT SERIES No. 250

**CHRONIC PULMONARY DISEASE IN  
SOUTH WALES COALMINERS**

**III.—EXPERIMENTAL STUDIES**

- A.—REPORT BY THE COMMITTEE ON INDUSTRIAL PULMONARY DISEASE**
- B.—THE MINERAL CONTENT OF THE LUNGS OF WORKERS FROM THE SOUTH WALES COALFIELD** by E. J. King and G. Nagelschmidt
- C.—THE ESTIMATION OF COAL AND OF ALUMINIUM IN DRIED LUNG** by E. J. King and Margaret Gilchrist
- D.—TISSUE REACTIONS PRODUCED EXPERIMENTALLY BY SELECTED DUSTS FROM SOUTH WALES COALMINES** by T. H. Belt and E. J. King
- E.—THE SOLUBILITY OF DUSTS FROM SOUTH WALES COALMINES** by E. J. King

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The late Dr. R. L. RAWLINSON was also a member of the Committee at the time of these investigations.

## PREFACE

This Report deals with the experimental section of the investigation of chronic pulmonary disease in South Wales coalminers which the Medical Research Council undertook in 1936 at the request of the Home Office and the Mines Department. It is complementary to two Reports already issued, one on the medical survey and related pathological studies, and the other on environmental studies—physical, chemical and petrological (*Spec. Rep. Ser. Med. Res. Coun., Lond.*, No. 243, 1942 and No. 244, 1943). The whole scheme of inquiry has been directed for the Council by their Committee on Industrial Pulmonary Disease, who have provided covering reports for each of the three publications, reviewing and summarizing the conclusions of the investigators. The Council are indebted to the members of the Committee—and particularly to the Secretary, Dr. E. L. Middleton, O.B.E.—for their labours.

This third publication completes the account of the investigations which have been made under the particular scheme. It is clear from the reports however, that many questions still remain unanswered and that further research of different kinds is needed for their elucidation. The Council have accordingly in view an extended programme of investigation, to be put in hand as soon as circumstances permit.

*2nd March, 1945.*

MEDICAL RESEARCH COUNCIL,  
c/o London School of Hygiene and Tropical Medicine,  
Keppel Street, London, W.C.1.

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## CHRONIC PULMONARY DISEASE IN SOUTH WALES COALMINERS.—III. EXPERIMENTAL STUDIES

### A.—REPORT BY THE COMMITTEE ON INDUSTRIAL PULMONARY DISEASE

1. This is the third Report on a series of studies directed by the Committee on Industrial Pulmonary Disease towards the elucidation of the problem of chronic pulmonary disease in the coalminers of South Wales. The first Report (*Spec. Rep. Ser. Med. Res. Coun., Lond.*, No. 243, 1942) dealt with the results of a medical survey and pathological examinations, and, simultaneously with the survey, investigations were made of environmental factors which may play a part in the causation of the disease. While the first Report showed that airborne dust must be regarded as the factor primarily responsible, the second (*Spec. Rep. Ser. Med. Res. Coun., Lond.*, No. 244, 1943) was directed to a consideration of the physical and chemical nature of the dust to which the miner was exposed, and to the chemical and petrological characteristics of the coal-seams and immediately adjacent rock strata from which the dust is derived. In their introductory report to the second monograph, the Committee suggested that the variations in the incidence of pneumokoniosis might depend on differences in the dusts, and they mentioned that some experiments had been begun to explore this possibility and to throw light on the relative pathogenicity of the different components of the dust, and of mixtures of these components.

The present Report includes accounts of the analysis of the mineral deposits found in the lungs of workers in the South Wales coalfield, of an experimental study of the tissue reactions produced in the lungs of experimental animals by selected dusts from the South Wales coalmines, and of a study of the solubility of such dusts.

2. In an extensive investigation, Professor King, Dr. Nagelschmidt and Mrs. Gilchrist have determined the composition and concentration of coal and mineral dust found at autopsy in the lungs of 54 coal workers. The composition of the lung residue varies for different occupational groups, in accordance with differences in the dust-producing strata encountered. In the lungs of anthracite colliers there was no significant increase in dust, total silica or quartz with increasing degrees of fibrosis. In the whole series of lungs, however, there was significantly more quartz in the silicotic nodulation category than in the less severe fibrosis groups. The investigators consider quartz, rather than mica or kaolin, to be the chief hazard, and they discuss the various ways in which the quartz effect might be so modified by the other dust constituents as to produce the lung changes observed.

3. In their experimental investigation of the effects on the lungs of rats of different specimens of dust obtained from the South Wales coalmines, Dr. Belt and Professor King have used the late Professor E. H. Kettle's method of introducing the dust in a single large dose directly into the trachea. A large number of mineral dusts and mixtures of dusts was studied in this way. In this connexion, it must be borne in mind that the experimental method employed takes no cognisance of the time factor, which may be of importance in the naturally occurring disease in man when dust has gradually accumulated in the lungs over a period of years. On the other hand, the exposure of

experimental animals to various dusts under conditions similar to those of workers in industries in which silicosis is known to occur is difficult and may equally be fraught with possible fallacies.

Dr. Belt and Professor King find that the purest coal dust, whether anthracite, steam coal or bituminous, has a low pathogenicity and causes only minimal fibrosis, but that the pathogenicity becomes greater as the content of siliceous material in the coal dust increases. Shale dust was found to be of low pathogenicity, and so were certain isolated "silicate dusts" consisting of fairly pure mica. The pathogenic action of quartz dust was substantially reduced if it was mixed with shale dust. When quartz dust was mixed with coal, the resultant confluent fibrosis was in distribution and type more like that seen in pneumokoniosis occurring in coal workers than that characteristic of the effect of pure quartz dust. The reasons for the pathogenicity of some siliceous dusts and the relative harmlessness of others are, however, by no means clear. Although the present investigation has given certain definite pointers, it is necessary that more experimental work should be done.

4. In recent years the deleterious effect of silica has been attributed to the toxic action which it can produce on the tissue cells when it is in solution. Professor King has investigated the solubility of silica in the different specimens of dust obtained from the South Wales coalmines, and the factors in such dusts which may depress the solubility below that characteristic of pure silica, particularly the effect of aluminium in various forms. But although it may be true in general that the more soluble a siliceous dust, the greater its toxicity, many variations from this generalization are shown in the South Wales mine dusts studied by Professor King. At least a partial explanation can be offered for these discrepancies, but it is again clear that a satisfactory answer must depend on further investigation.

5. The results of the medical studies described in the Committee's first Report raised two important questions:—

- (i) What are the cause and the mechanism of production of the pneumokoniosis of coal workers, and what is its relation to "classical" silicosis?
- (ii) Why does the incidence of pneumokoniosis amongst colliers in South Wales decrease with the rank of the coal mined, from high in anthracite to low in bituminous coal?

The data presented in the present and previous Reports suggest that, while quartz is probably the most noxious factor, the difference between this pneumokoniosis and "classical" silicosis (as seen, for example, in sandstone workers) is due to small amounts of quartz being mixed, in the former case, with large amounts of coal and other minerals, as has, indeed, been suggested by others who have studied the problem. The mechanism of the interaction of quartz with other silicates and with coal—and the possible antidotal effects of aluminium, iron, etc.—require further study, which the Committee regards as a matter of great importance.

The relationship of incidence of pneumokoniosis to rank of coal might be due to a generally higher dust concentration in anthracite mines, to differences of interaction of quartz with different types of coal, or to differences of the modification of the quartz effect, due to differences in the mineral composition of the shales and other strata associated with the coal. The published Reports show that all these effects may be involved in causing the differences in pneumokoniosis incidence. But despite the great amount of information elicited, many doubts and discrepancies have still to be dispelled, and the Committee feel

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that it would be imprudent at this juncture to draw any firm conclusions. They agree with those who have carried out the investigations, that much further study is necessary of the reaction of the lungs of animals to the different components of mine dust, tested both individually and in different combinations, as well as of the factors which affect the solubility of silica and silicates in the body fluids; and that this information must be correlated with the features which characterize pneumokoniosis as it develops in man under the every-day conditions of his work.

6. The Committee wish to thank the investigators for the care and skill with which they have carried out these laborious researches. They also wish to express their high appreciation of the work of their Secretary—Dr. E. L. Middleton, O.B.E.—which has proved invaluable throughout the whole inquiry covered by the three Reports; the constant and critical alertness which he has maintained, together with his great experience, have overcome many of the difficulties which the Committee would have otherwise encountered, and have enabled the investigations to be continued in spite of the handicap imposed by the war.

The Committee have learnt with very great regret of the untimely death of Dr. T. H. Belt, which occurred while this Report was in the press. The Committee owe much to him, and his work published in the first of the three Reports, and in the present monograph, has formed a very important contribution to the study of the pneumokoniosis of coal workers. It is indeed regrettable that he should not have lived to continue the experimental work which he had developed with such ability.



# CHRONIC PULMONARY DISEASE IN SOUTH WALES COALMINERS.—III. EXPERIMENTAL STUDIES

## B.—THE MINERAL CONTENT OF THE LUNGS OF WORKERS FROM THE SOUTH WALES COALFIELD

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### INTRODUCTION

The pulmonary abnormalities found in the South Wales coalmining population were described in detail by Hart and Aslett (1942) and a description of the pulmonary changes in 42 miners has been given by Belt and Ferris (1942). The present report deals with the analytical results obtained in a study of 54 lung residues, 40 of which were obtained from the cases described by Belt and Ferris.

The main object of the work was to obtain quantitative information about the amount and composition of the dust found in the lungs, and, in particular, about the amount and type of combination of the silica. It is well known that silicotic lung tissues always contain abnormally large amounts of silica. The converse, however, is not always true, i.e., the presence of excessive amounts of silica in the lungs and lymph nodes is not invariably associated with the presence of silicotic lesions (King and Belt, 1940; Gardner and Redlin, 1942). Many lungs which come from men exposed for years to siliceous dust, and which contain large amounts of silica, show no evidence of having developed silicotic nodules, the characteristic lesion of the disease.

The inhalation of silica-containing dust, then, may or may not produce silicosis. There are probably two groups of factors causing this variation, (a) biological factors, including individual susceptibility and the presence or absence of infection (Kettle, 1932), (b) differences in dust content and composition. The same amount of total silica in a given dust may be present in very different forms, and may be accompanied by other constituents which modify its action. It is likely that the two groups always operate together, but our work contributes information about the second only, i.e., the amount and composition of the dust in the lungs.

## 2 PULMONARY DISEASE IN SOUTH WALES COALMINERS

Silica occurs in nature, and in air-borne dusts, in different forms. It is customary to distinguish between "free" silica and "combined" silica. In "free silica" the minerals quartz, tridymite and cristobalite are grouped together with amorphous silica (flint, for instance, is a mixture of quartz—sometimes cristobalite—and amorphous silica); combined silica comprises all silicates, such as mica, kaolin, asbestos, etc. Quartz and flint are currently thought to form dangerous dusts, since industrial experience shows a high incidence of silicosis amongst workers exposed to them. For similar—though not so well-founded—reasons, mica and kaolin are often regarded as being relatively innocuous. Experiments with animals have, to a large extent, confirmed these views. Thus it would be possible to have equal concentrations of "silica" in two dusts, constituting quite different hazards. But most industrial dusts are mixtures of several forms of silica with or without other non-siliceous constituents. In order to arrive at an understanding of the aetiology of dust disease, it is therefore not sufficient to know only the silica content of any given dust: one must know all components and their concentrations.

Reputedly harmless constituents of dust may not only act as diluents of the harmful constituents, but may modify or inhibit their action. Haldane (1931) believed that shale dust modified the action of quartz in the lung, and indeed acted as a natural antidote to the free silica (see also Gardner and Durkan, 1938). Later it was shown that shales and many pure silicates would markedly decrease the rate at which quartz dissolved in body fluids (Whitehouse, 1938; King and Roman, 1938) and that free alumina and metallic aluminium would almost completely depress silica solubility (Denny, Robson and Irwin, 1939). A study of the mineral content of lungs derived from a restricted industrial area, where the dust breathed contained the same components and differed only in regard to their relative proportions might, therefore, throw some light on why certain men develop a severe pneumokoniosis while others with equally long exposures develop a milder form of lung affection.

The dusts breathed by workers in the South Wales coalmines may contain quartz, mica, kaolin, carbonates and other non-siliceous constituents in minor amounts, in addition to coal (cf. *Spec. Rep. Ser. Med. Res. Coun., Lond.*, No. 244, 1943). The amounts of the various constituents may vary in the dusts to which different occupational groups are exposed, and such variations may be reflected in the dusts isolated from lungs. It is possible, moreover, that the relative proportions of aluminous minerals and of quartz may be of greater aetiological significance than the concentration of any one constituent. If the antidotal rock theory is valid, then the more kaolin and mica there are relative to quartz, the less is the chance of nodular fibrosis developing. Certain indications that this may be the case in experimental pneumokoniosis have been found by Belt and King in the animal experiments forming part of this investigation (p. 61 below). The powdered clod strata of the South Wales mines are largely mixtures of quartz, mica and kaolin, with mica predominating and certain other minor constituents present in small amounts. These clods have produced the minimum of fibrotic reaction in animal lungs, and when mixed with quartz have appeared to suppress its fibrogenic properties. Moreover, when quartz pebbles are immersed in water containing these powdered clods, they become coated with a layer of alumina and stain a bright red with aurine, as in the experiments of Denny, Robson and Irwin (1939) with quartz and powdered aluminium metal. It seemed, therefore, that the  $\text{Al}_2\text{O}_3/\text{SiO}_2$  ratios in the siliceous lung residues would be worth determining directly, as well as the concentrations of the individual minerals.

The material studied comprised lung specimens from 27 anthracite colliers, 6 steam coal colliers, 2 "rock" colliers (i.e., colliers in certain non-anthracite

mines, handling sandstone strata when ripping), 5 rock workers (hard-heading men, borers and repairers), 4 firemen or shotmen, 4 men having miscellaneous underground occupations, 2 workers on surface screens and 4 coal trimmers at docks. The pathological investigations led Belt and Ferris (1942) to distinguish five different histological types of lung change by the terms *reticulation (or dust reticulation)*, *reticular*, *mixed and silicotic nodulation*, and *confluent fibrosis*. As several of these terms were innovations in the terminology of histological changes in the lung it is advisable to recapitulate here their meanings. They are as follows:—

*Reticulation or dust reticulation*: a proliferation of reticulin fibrils, enclosing dust cells, of streaky distribution in the lung. It was the histological change found in lungs showing reticulation in radiographs, and was applied to the proliferation of reticulin fibrils to emphasize that such proliferation is the histological characteristic of the X-ray picture. Unfortunately, this term led to some confusion and criticism (Tyter, 1944; Williams, 1944).

*Reticular nodulation*: focal indurations, rounded, angular, polygonal or stellate, composed of reticulin fibrils enclosing dust cells.

*Mixed nodulation*: similar focal indurations containing in addition to reticulin fibrils a greater or less amount of hyaline collagenous fibres, particularly in their centres.

*Silicotic nodulation*: typical silicotic nodules composed of hyaline collagenous fibres, which tend to be concentrically arranged and the outer to be woven into a dense, sharply defined collagenous capsule.

*Confluent fibrosis*: areas formed by the confluence of dust reticulation. There may be in addition one or more of the other changes described above; the confluence is accompanied by the appearance of added collagenous fibrosis in a greater or less degree.

In this report the term *mixed nodulation* is used to include both *reticular nodulation* and *mixed nodulation*. It seemed possible that the five different types of histological change of Belt and Ferris, now treated as four, might be reflected in the amount and composition of the dust in the lungs.

## METHODS

The method of separating the mineral matter from the lungs has been fully described elsewhere (Nagelschmidt and King, 1941). In brief, it consists of  $H_2O_2$  digestion of the powdered, dried, formalinized lung, followed by extraction with dilute acid to remove the endogenous salts deriving from the lung tissue. The insoluble residue, consisting of coal and siliceous minerals, is separated and washed with water by centrifuging. The coal is removed by prolonged low temperature combustion ( $380^\circ C.$ ). At a higher temperature, the layer lattice of kaolin would be destroyed. The  $380^\circ$  ash of the residue is submitted to chemical and X-ray diffraction pattern analysis, for identification of the minerals present, and for assessment of their relative amounts. In the case of some of the lungs, the  $H_2O_2$  digestion has been omitted, the dry powdered lung being ashed at  $380^\circ C.$ , and the resultant mixture of siliceous minerals and salts extracted with cold, dilute HCl to remove the salts. The percentage of "coal" is determined on another sample of dried lung, by a double extraction with hot soda solution, which destroys the tissue without appreciably altering the coal (King and Gilchrist, pp. 21–28 below). Determinations of silica were made by the method of King (1939), and of aluminium by Berg's (1935) hydroxyquinoline procedure.

The alumina to silica ratios were derived from analyses of the  $H_2O_2$ —residues, which were carried out in part by spectrographic analysis (by Miss T. Strookman, B.Sc.) and in part by chemical analysis (by Mrs. M. Gilchrist, M.Sc.). As the residues were not equally free of non-siliceous matter, i.e. endogenous lung salts, the percentages of  $Al_2O_3$  and of  $SiO_2$  cannot profitably be compared from one lung residue to another. But the ratios of alumina to silica are independent of residual lung material, and it is these which are compared in the different pathological groups.

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As described in a previous report (Nagelschmidt, 1943), the ash of 25 air-borne dusts has been analyzed chemically, and the amounts of quartz, kaolin and mica calculated as percentages of the sum of these three minerals. By relating these percentages to the X-ray diffraction estimates (high, medium, low, etc.) of the three minerals in the acid-treated ash of the dust, it is possible to substitute percentage figures for the X-ray estimates. The lung residue ashes are comparable, in mineral content and in the method of preparation, with the dust ashes, and the same substitution can, therefore, be made. As the X-ray estimates are based on visual comparisons of the relative intensities of diffraction lines, they are not very accurate, and consequently the percentages of quartz, mica and kaolin obtained are only approximations; for instance, they do not always add up to 100. They do, however, indicate the average lung dust composition in terms of the siliceous minerals found.

In order to correlate the results with the pathological findings, it is necessary to consider not only the relative composition of the dusts in the lungs, but also the absolute amounts of the various minerals, and of the coal, in the different lungs. The amounts of quartz, kaolin and mica per lung cannot be determined simply by multiplying the percentages derived from X-ray data by an ash percentage for each lung. The  $H_2O_2$  treatment, used to isolate the siliceous minerals, yields a residue which may contain non-siliceous components (due to their incomplete extraction by cold, dilute acid), and the ash percentage of the  $H_2O_2$  residues would therefore be too high. On the other hand, the soda treatment, used to extract the coal, removes some of the siliceous minerals, and the resulting ash percentages would be too low. The estimation of quartz, mica and kaolin per lung was therefore based on the chemical determination of the total  $SiO_2$  per lung. This was allocated to the three minerals according to their proportions as indicated by X-ray diffraction analysis. The percentage of quartz in the total silica was calculated from the percentage of quartz in the siliceous minerals, on the assumption that mica and kaolin both contained 45 per cent. of  $SiO_2$ . After the resulting amount of quartz had been subtracted from the total  $SiO_2$ , the remainder was divided between mica and kaolin, according to their estimated proportions, and their amounts were found by multiplication with 2.2. The sum of quartz, kaolin and mica per lung, derived in this way, and the amount of coal per lung from the soda treatment, were used to calculate the coal and mineral percentages of the dusts in the lungs.

### RESULTS

#### EFFECT OF OCCUPATION ON LUNG-DUST COMPOSITION

The full data are set out in Table I, where the results are grouped according to the chief occupations of the subjects and, within the occupational groupings, according to the pathological findings. They show a relation between the chief occupation and the composition of the dust found in the lungs. The average results for the different occupations are given as percentages of the lung-dust in Table II. It will be seen that the lungs of trimmers and screen workers had the highest proportion of coal and the lowest proportion of siliceous minerals; those of rock workers, on the other hand, had only a little coal and a high proportion of siliceous minerals. The lungs of colliers, both in anthracite and steam coal, occupied an intermediate position, but their lung dust was more like that of the trimmers than that of the rock workers. As the amount of coal increased, the proportion of quartz and of mica fell and that of kaolin increased. This is in agreement with the results of the South Wales strata investigation (Hicks and Nagelschmidt, 1943), according to which kaolin is the main siliceous mineral of the coal seam mineral matter, whereas quartz and mica are the main constituents of the rocks.

TABLE I  
Mineral Content of Lungs of Coalminers

Chief occupation.	P.M. No.	Duration of exposure (years). <sup>a</sup>			Patho- logical grouping. <sup>†</sup>	X-ray. <sup>‡</sup>	Silica. (figures represent percentages by weight of the dried lung).	Coal.	Siliceous Minerals.	Quartz.	Mica.	Kaolin.
		1	2	3								
I. Rock workers .. ..	176	28		1	4	e	1.99	1.1	3.54	0.72	1.31	1.51
	181	28		2	4	e	1.75	1.3	3.00	0.73	1.45	0.82
	159	14	5 IV	2	5	d	1.60	0.7	2.60	0.78	0.78	1.04
	166	20		7	5	c	1.06	1.1	1.72	0.52	1.02	0.18
	170	16		2	5	d	4.35	0.4	7.77	1.57	2.90	3.30
II. Rock colliers .. ..	150	25	8 VI	2	2	b	1.22	2.9	1.97	0.61	0.65	0.71
	157	11	7 VI	3	4	d	0.86	3.5	1.40	0.42	0.47	0.51
III. Firemen or shotsmen ..	169	28	17 V		3	c	1.33	6.5	2.51	0.37	0.89	1.25
	121	23	13 V		3	d	1.53	5.4	2.87	0.43	0.91	1.53
	132	16	19 V		4	e	1.66	9.6	3.12	0.47	1.00	1.65
	151	20	7 VI		4	e	1.29	13.7	2.43	0.36	0.80	1.27
IV. Miscellaneous—haulers, repairers, roadmen— working underground.	161	39		5	2	a, b	1.02	16.8	1.83	0.36	0.60	0.87
	155	30			2	a	0.61	1.9	1.15	0.17	0.62	0.36
	141	31			2	b	1.76	15.0	3.32	0.49	1.16	1.67
	128	51		1	3	b	1.42	13.9	2.67	0.40	1.56	0.71
V. Anthracite colliers ..	115	35			2	b	1.39	14.6	2.62	0.39	0.78	1.45
	135	27	14 VI	1	2	b	1.18	24.9	2.22	0.33	0.62	1.27
	145	34	9 VI		2	b	1.06	7.3	2.10	0.21	0.67	1.22
	147	32	10 IV	3	2	b	0.94	17.3	1.86	0.19	0.65	1.02
	154	42			2	b	0.97	9.4	1.83	0.27	0.56	1.00
	190D	45		8	2	b, c	0.43	5.5	0.81	0.12	0.27	0.42
	191B	41	9 VI	5	2	b	0.70	11.0	1.31	0.20	0.42	0.69
	191E	31			2	b	0.93	12.2	1.75	0.26	0.67	0.82
	123	24			3	e	1.40	13.2	2.64	0.39	0.76	1.49
	152	28	7 VI	4	3	d	1.35	7.3	2.67	0.27	0.93	1.47
	162	35	9 VI	6	3	d	1.25	13.0	2.35	0.35	0.78	1.22
	163	34	11 VI	3	3	f	1.42	14.5	2.67	0.40	0.85	1.42
	168	17	6 IV	3	3	c	1.36	16.1	2.56	0.38	1.02	1.16
	177	36	11 IV	5	3	d	0.72	13.0	1.43	0.14	0.51	0.78
190B	—	—	9	3	c?	1.14	9.0	2.14	0.32	0.71	1.11	

MINERAL CONTENT OF THE LUNGS

TABLE I—contd.  
Mineral Content of Lungs of Coalminers

Chief occupation.	P.M. No.	Duration of exposure (years).*			Pathological grouping †	X-ray ‡	Silica. (figures represent percentages by weight of the dried lung).	Coal.	Siliceous Minerals.	Quartz.	Mica.	Kaolin.
		1	2	3								
	175	23		2	4	e	1.62	26.0	3.05	0.45	1.22	1.38
	178	33		4	4	e	1.02	16.9	1.91	0.29	0.62	1.00
	179	21		5	4	e	0.86	23.8	1.53	0.31	0.58	0.64
	180	36		6	4	e	1.57	15.1	2.96	0.44	0.98	1.54
	183	34		5	4	e	0.82	7.3	1.54	0.23	0.51	0.80
	184	23		7	4	e	2.07	13.3	3.89	0.58	1.29	2.02
	185	38		4	4	e	0.88	18.9	1.66	0.25	0.47	0.94
	190W	54		9	4	—	1.11	7.8	2.09	0.31	0.69	1.09
	190Sa	26		8	4	d	0.64	23.6	1.20	0.18	0.40	0.62
	190D1	40		8	4	d	0.93	18.9	1.75	0.26	0.62	0.87
	190M	47		8	4	e	0.83	8.9	1.56	0.23	0.51	0.82
	190R	46		13	4	e	0.82	12.2	1.54	0.23	0.51	0.80
VI. Steam coal colliers ..	191H	29	10	2	2	a, b	0.76	5.0	1.43	0.21	0.47	0.75
	153	37	12 V	3	3	b	1.55	7.1	2.92	0.43	0.98	1.51
	167	31		2	3	d	1.56	20.9	2.93	0.44	1.02	1.47
	156	20		2	4	e	1.17	12.3	2.20	0.33	0.78	1.09
	158	26		3	4	e	3.01	14.2	5.65	0.84	2.00	2.81
	160	36		2	4	e	1.61	16.8	3.03	0.45	1.00	1.58
VII. Screen workers ..	117	21			4	e	1.30	16.5	2.45	0.36	0.62	1.47
	133	20			4	e	0.95	26.6	1.78	0.27	0.53	0.98
VIII. Coal trimmers ..	Gr	28			2	b	0.23	6.1	0.43	0.06	0.13	0.24
	Pi	30			3	c, d	0.25	8.6	0.45	0.07	0.11	0.27
	Mi	25		9	4	e	0.44	20.8	0.83	0.12	0.22	0.49
	174	39			4	e	0.30	12.8	0.57	0.08	0.20	0.29

\*Duration of exposure :—1, years spent in stated occupation ; 2, years spent in other underground occupation (IV = miscellaneous, V = anthracite collier, VI = non-anthracite collier) ; 3, years between removal from dust and death.  
 † Pathological grouping :—2 = reticulation ; 3 = reticular and mixed nodulation ; 4 = confluent fibrosis ; 5 = silicotic nodulation.  
 ‡ X-ray :—a = normal ; b = reticulation ; c = nodulation ; d = coalescent nodulation ; e = massive shadows ; f = multiple fluffy shadows.  
 cf. Hart and Aslett (1942).

(87082)

PLATE I



X-ray powder diagrams of (a) quartz, (b) a rock worker's lung residue, (c) an anthracite collier's lung residue ; (b) shows weak and (c) strong non-quartz lines.

PLATE II

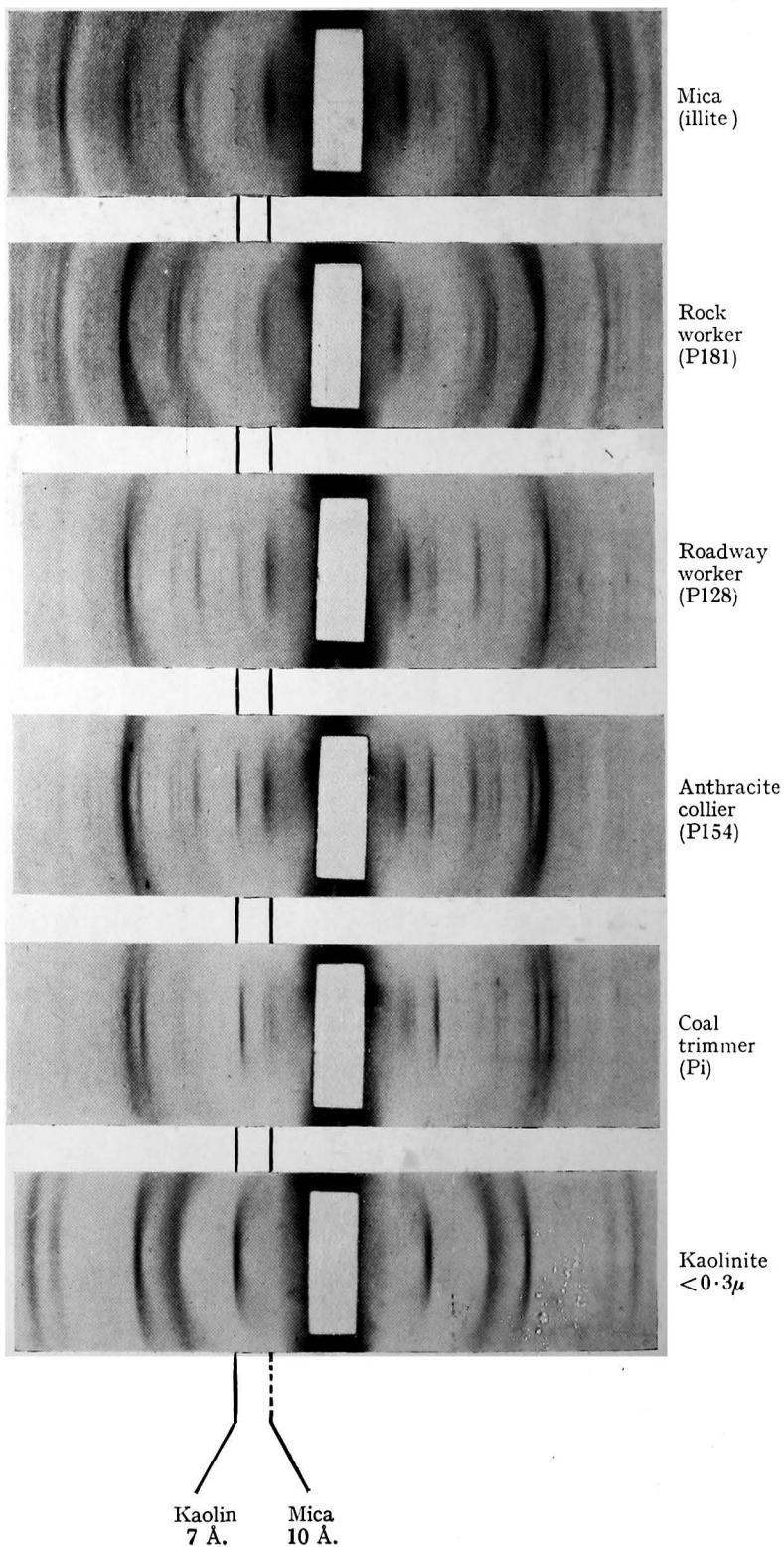




TABLE II

*Average Composition of Dust from Lungs of Different Occupational Groups*

	Coal. (As percentage of dust from lungs.)	Siliceous Minerals.	Quartz. (As percentage of siliceous minerals from lungs.)	Mica.	Kaolin.
Rock workers (5)* .. ..	25	75	25	40	30
Rock colliers (2) .. ..	66	34	30	35	40
Firemen or shotsmen (4) .. ..	76	24	15	35	50
Miscellaneous workers (4) .. ..	76	24	15	50	35
Anthracite colliers (27) .. ..	86	14	15	35	50
Steam coal colliers (6) .. ..	85	15	15	35	50
Screen workers and coal trimmers (6).	95	5	15	25	55

\* Figures in brackets refer to the number of cases studied.

This is further illustrated by the X-ray diagrams given in Plates I and II. Plate I shows powder diagrams of (a) quartz, (b) a rock worker's and (c) an anthracite collier's lung residue; (b) shows weak and (c) stronger non-quartz lines. Plate II shows aggregate diagrams of lung residues from a rock worker, a roadway worker, an anthracite collier and a trimmer. M and K indicate mica and kaolin lines and, in the above order, the mica lines decrease and those of kaolin increase in relative intensities.

Table III shows the average results for lung residues from men in different occupations, expressed as percentages of the dry weight of the lung. Standard errors were calculated and values significantly different (P under 0.05 by t-test) from those for anthracite colliers are underlined. It will be seen that the lungs of rock workers have twice as much total silica and mica, and nearly three times as much quartz, as those of anthracite colliers; they have only slightly more kaolin and much less coal. The lungs of trimmers, on the other hand, have significantly less quartz, mica and kaolin, than those of anthracite colliers, but it appears that they do *not* have more coal. More lungs of trimmers should be examined to confirm this point.

#### COMPARISON OF DUST IN LUNGS WITH AIR-BORNE DUST

The composition of the siliceous minerals in the lungs of anthracite colliers (Table II) is: quartz 15 per cent., mica 35 per cent. and kaolin 50 per cent. This may be compared with the average composition of the air-borne dusts at the coalface in anthracite mines (*Spec. Rep. Ser. Med. Res. Coun. Lond.*, No. 244, p. 120), viz., quartz 12 per cent., mica 35 per cent., kaolin 53 per cent.

The mineral percentages show that the relative amounts of the siliceous minerals are very similar, implying either that none of these minerals is dissolved or altered to any large extent, or that they are all dissolved at the same rate, so that their relative proportions do not vary. The ash percentage of the air-borne dusts is 10 per cent. and that of the lung residues 14 per cent. As the ash figure for the lung residues is obtained in an indirect way, the difference is not considered sufficiently large to merit attention; on the contrary, the similarity of the values appears significant.\*

This similarity between air-borne and lung-retained dusts is not due to the fact that fresh dust is constantly added to the lungs and masks any change in mineral composition. The 27 anthracite colliers whose lungs were examined had all been exposed to dusts of similar composition. They can be grouped

\* The differences in composition observed here between air-borne dust and lung dust are much smaller than those found by Gardner and Durkan (1938), who studied dust composition in an experimental dusting room and in the lungs of animals breathing the dust.

TABLE III  
*Mineral Content of Lungs in Relation to Occupation*  
*(Mean values and their standard errors)*

<i>Principal occupation.</i>	<i>No. of cases.</i>	<i>Years exposure.†</i>			<i>Total Silica.</i>	<i>Coal.</i> <i>(figures represent percentages by weight of dry lung).</i>	<i>Siliceous minerals.</i>	<i>Quartz.</i>	<i>Mica.</i>	<i>Kaolin.</i>
		(1)	(2)	(3)						
I. <i>Rock workers</i> .. ..	5	21	1	3	<u>2.15</u> (±0.57)‡	<u>0.92</u> (±0.16)	<u>3.73</u> (±1.04)	<u>0.86</u> (±0.18)	<u>1.49</u> (±0.37)	<u>1.37</u> (±0.53)
II. <i>Rock colliers</i> .. ..	2	18	8	2	1.04	3.2	1.68	0.52	0.56	0.61
III. <i>Firemen and shotmen</i> ..	4	22	14	—	1.45 (±0.09)	8.8 (±1.9)	2.73	<u>0.41</u> (±0.03)	0.90	1.42
IV. <i>Miscellaneous*</i> .. ..	4	35	—	2	1.20	11.9	2.24	0.35	0.98	0.90
V. <i>Anthracite colliers</i> .. ..	27	34	5	5	1.09 (±0.07)	14.1 (±1.1)	2.06 (±0.13)	0.30 (±0.02)	0.69 (±0.05)	1.08 (±0.07)
VI. <i>Steam coal colliers</i> ..	6	30	4	2	<u>1.61</u> (±0.31)	12.7	<u>3.03</u> (±0.58)	<u>0.45</u> (±0.09)	1.04	1.51
VII. <i>Screens workers</i> .. ..	2	20	—	—	1.12	21.5	2.12	0.32	0.58	1.22
VIII. <i>Trimmers</i> .. ..	4	30	—	—	<u>0.31</u> (±0.05)	12.1	<u>0.57</u> (±0.09)	<u>0.08</u> (±0.01)	<u>0.16</u> (±0.03)	<u>0.32</u> (±0.06)

N.B.—Underlined values are significantly different from the corresponding values for anthracite colliers.

\* Miscellaneous: hauliers, repairers, roadway men, working underground.

† Years exposure: (1) years in stated occupation; (2) years in other underground occupation; (3) years between removal from dust and death.

‡ (±.....) = Standard error of the mean.

according to the number of years that elapsed between removal from dust (work) and death. The relative proportions of siliceous mineral and the Coal/SiO<sub>2</sub> ratios of the average dust in the lungs of the three groups are as follows :—

<i>Years removed from occupation.</i>	<i>No. of Cases.</i>	<i>Quartz.</i>	<i>Mica.</i>	<i>Kaolin.</i>	<i>Coal/SiO<sub>2</sub> Ratio.</i>
		<i>(percentage of total minerals.)</i>			
Under 3 .. ..	10	14	32	53	13
4-6 .. ..	9	15	35	54	15
7-13 .. ..	8	15	35	54	13

Thus, if any changes in mineral content occur in the lungs, they are too small to be demonstrable by the analytical technique we used. There is, however, some evidence of a reduction in the dust concentration in the lungs as the time interval between removal from exposure to dust and death increases (see p. 13 below).

#### COMPARISON OF DATA WITH PATHOLOGICAL FINDINGS

##### (a) All Cases

Table IV gives the average results for mineral content obtained for the 54 lungs examined. They are arranged according to the groups in the histological classification. The number of years during which the men had been exposed to the dust was great in nearly all cases, and in the groups where less fibrosis of the lungs had developed the duration of exposures was as long as in those where there was well developed nodulation. These lungs would therefore seem to provide a proper basis for comparing the dust content of lungs showing the different types of fibrosis, despite the fact that the dust had been present in the lungs of the different groups for equally long periods. The figures for coal show the most marked contrast in Table IV. There was less than 1 per cent. in the lungs of those who developed silicotic nodulation, and over 10 per cent. in the other groups. But this is, of course, a reflection of the occupational exposure. The 3 men with silicotic nodulation were workers in rock, and the 2 rock workers who developed confluent fibrosis (Nos. 176 and 181 in Table I) had similar small amounts of coal in their lungs. Likewise, other subjects, whose work had been largely in rock, showed small concentrations of coal in the lungs. Such lungs are found in all the pathological groupings.

The total silica figures, and those for the siliceous minerals, show a gradual increase on passing from reticulation through mixed nodulation and confluent fibrosis to silicotic nodulation. The percentage of kaolin is, on the average, much the same in all groups. The quartz and mica are appreciably less in the reticulation than in the mixed nodulation and confluent fibrosis groups; in the silicotic nodulation group, they are considerably greater. There is 20 and 34 per cent. more quartz and 43 and 32 per cent. more mica in the mixed nodulation and confluent fibrosis groups than in the reticulation group, and silicotic nodulation has on the average 3½ times as much quartz and 2½ times as much mica as has reticulation.

The usual statistical test of the significance of differences between the average figures is shown in Table IVA. The mixed nodulation group contains more mica and kaolin than the reticulation group. This is reflected in the total silica figure. Quartz and coal are not significantly different. There is no significant difference between confluent fibrosis and either reticulation or mixed nodulation. Silicotic nodulation, on the other hand, shows significant contrasts with all other groups. The differences in non-coal minerals are greatest in the case of quartz, and they increase as the pathological contrast becomes greater. Thus, there is a difference of 0.58 per cent. of quartz

TABLE IV  
Average Mineral Content of Lungs (all cases) in Relation to Pathological Findings

Pathological group.	No. of cases.	Years exposure.*		Total silica. per cent. †	Coal. per cent.	Siliceous minerals. per cent.	Quartz. per cent.	Mica. per cent.	Kaolin. per cent.
		1 and 2	3						
(2) Reticulation .. .. .	14	38	2	0.94 (±0.11) †	10.7 (±1.7)	1.76 (±0.19)	0.28 (±0.04)	0.59 (±0.07)	0.89 (±0.11)
(3) Reticular and mixed nodulation	13	38	3	1.25 (±0.10)	11.4 (±1.24)	2.37 (±0.19)	0.34 (±0.03)	0.85 (±0.091)	1.18 (±0.08)
(4) Confluent fibrosis .. ..	24	31	4	1.23 (±0.12)	14.2 (±1.5)	2.28 (±0.23)	0.37 (±0.04)	0.78 (±0.09)	1.12 (±0.11)
(5) Silicotic nodulation .. ..	3	18	4	2.34 (±1.02)	0.7 (±0.2)	4.03 (±1.88)	0.96 (±0.32)	1.57 (±0.67)	1.51 (±0.94)
Whole series .. .. .	54	34	3	1.22 (±0.09)	11.9 (±0.94)	2.26 (±0.16)	0.37 (±0.03)	0.79 (±0.06)	1.10 (±0.08)

\* Years exposure:—1 and 2, years occupied; 3, years between removal from dust and death.

† (±....) = standard error of the mean.

‡ Figures represent percentages by weight of the dried lung.

TABLE IVA  
Difference in Mineral Content in Pathological Groups (all cases)

Contrast.	Total Silica. (Differences between means, with their standard errors ( $\pm \dots$ )†, and t tests, i.e. difference between means $\div$ standard error of the difference, expressed as probabilities, p, that the differences between means are due to chance.)	Coal.	Quartz.	Mica.	Kaolin.
Mixed nodulation* minus reticulation, i.e. (3)–(2).	0.310 ( $\pm 0.153$ ) p=0.1–0.05	0.72 ( $\pm 2.08$ ) >0.5	0.061 ( $\pm 0.051$ ) 0.5–0.1	0.257 ( $\pm 0.116$ ) 0.05–0.02	0.292 ( $\pm 0.139$ ) 0.02–0.01
Confluent fibrosis minus reticulation, i.e. (4)–(2).	0.286 ( $\pm 0.182$ ) p=0.5–0.1	3.54 ( $\pm 2.36$ ) 0.5–0.1	0.095 ( $\pm 0.060$ ) 0.5–0.1	0.191 ( $\pm 0.124$ ) 0.5–0.1	0.233 ( $\pm 0.170$ ) 0.5–0.1
Silicotic nodulation minus reticulation i.e. (5)–(2).	1.394 ( $\pm 0.556$ ) p=0.05–0.02	–9.97 ( $\pm 4.37$ ) 0.05–0.02	0.681 ( $\pm 0.219$ ) <0.01	0.976 ( $\pm 0.370$ ) 0.02–0.01	0.615 ( $\pm 0.443$ ) 0.5–0.1
Silicotic nodulation minus mixed nodulation, i.e. (5)–(3).	1.085 ( $\pm 0.497$ ) p=0.05–0.02	–10.69 ( $\pm 3.64$ ) 0.02–0.01	0.619 ( $\pm 0.149$ ) >0.01	0.718 ( $\pm 0.365$ ) 0.10–0.05	0.323 ( $\pm 0.310$ ) 0.5–0.1
Silicotic nodulation minus confluent fibrosis, i.e. (5)–(4).	1.108 ( $\pm 0.497$ ) p=0.05–0.02	–13.42 ( $\pm 4.82$ ) 0.01	0.585 ( $\pm 0.181$ ) <0.01	0.784 ( $\pm 0.343$ ) 0.05–0.02	0.382 ( $\pm 0.372$ ) 0.5–0.1

† Standard error of difference between means =  $\sqrt{\frac{\sigma^2}{n_1} + \frac{\sigma^2}{n_2}}$ , where  $\sigma$  is the standard deviation of all cases in the groups compared, and  $n_1$  and  $n_2$  are the numbers of cases in each group.

Note.—Underlined values are significant by t test; i.e. the probability of their occurring by chance is less than 0.05 (1 in 20).

\* "Mixed nodulation" includes reticular nodulation.



between silicotic nodulation and confluent fibrosis, and of 0.68 per cent. between silicotic nodulation and reticulation, with an intermediate figure of 0.62 per cent. for mixed nodulation. Mica is likewise significantly different, but not so markedly as quartz. These differences are again reflected in the figures for total silica.

The ratios of alumina to silica are given in Table V. No great contrast was observed. It is true that there is less alumina relative to silica in the silicotic nodulation group, but the differences are not great; and the confluent fibrosis group actually had a higher ratio than the reticulation group, when the reverse might have been expected. These determinations of the relative concentrations of alumina and silica have, therefore, not yielded any useful information in elucidating the South Wales pneumokoniosis.

(b) *Anthracite Colliers*

The largest occupational group in this series of cases is that of anthracite colliers. The variability of exposure, both qualitatively and quantitatively, is less here than among the cases as a whole. A comparison of the mineral content of anthracite colliers' lungs having mixed nodulation or confluent fibrosis with those showing reticulation is given in Tables VI and VIa. The comparison of mineral content may be simplified by combining the mixed nodulation and confluent fibrosis groups, and contrasting the average figures with those for lungs diagnosed as reticulation. This makes a better basis for comparison, since the rather large variations among the figures within groups assume less importance among the larger number of lungs in the combined group. The average figures, together with their standard errors, are included in Tables VI and VIa. There is no significant contrast in this comparison. The percentage of mica is appreciably greater in mixed nodulation than in reticulation, but this difference is largely lost in the combined group. Although there is a little more of both quartz and mica in the combined fibrosis group than in reticulation, the differences lack significance. Coal and kaolin percentages are not appreciably different.

If the anthracite colliers' lungs shown in Table V be grouped in each pathological category according to the number of years which had elapsed between the individual's retiring from work and his death, then the following values for total  $\text{SiO}_2$  are seen:—

Pathological category.	Years removed from occupation.		All cases.
	Under 5.	Over 5.	
Reticulation .. ..	1.08	0.57	0.95
Mixed nodulation ..	1.38	1.04	1.23
Confluent fibrosis ..	1.17	1.07	1.10

This shows clearly a reduction in total silica upon removal from dusty work.

Dr. E. Lewis-Faning kindly made a statistical analysis of the figures for total silica in the lungs of anthracite colliers. He observed a negative correlation with the number of years removed from work (corr. coeff.=0.28) and a significant negative correlation with the number of years worked (corr. coeff.=0.43), i.e., the longer the working life had been, the less silica there was in the lungs. The correlation between years worked and years of retirement from work before death, on the other hand, was positive.

This may be taken as an indication of the action of biological factors. Less susceptible workers might be expected, in a given number of years, to retain less silica than more susceptible workers, and the latter would be apt to die more promptly than the former. The group of 27 anthracite colliers examined is too small to allow of any certain conclusions to be drawn. Lungs become available for analysis only in cases where a post-mortem examination is made, i.e., because of accidental death or arising out of a compensation claim. It

TABLE VI  
Average Mineral Content of Lungs (Anthracite Colliers) in Relation to Pathological Findings

Pathological group.	No. of cases.	Years exposure.*		Total silica. Per cent.‡	Coal. Per cent.	Siliceous minerals. Per cent.	Quartz. Per cent.	Mica. Per cent.	Kaolin. Per cent.
		1 and 2.	3.						
(2) Reticulation .. .. .	8	41	2	0.95 (±0.10)†	12.8 (±2.2)	1.81 (±0.23)	0.246 (±0.030)	0.58 (±0.057)	0.99 (±0.117)
(3) Reticular and mixed nodulation	7	36	4	1.23 (±0.09)	12.3 (±1.21)	2.35 (±0.18)	0.321 (±0.037)	0.79 (±0.065)	1.24 (±0.099)
(4) Confluent fibrosis .. .. .	12	35	7	1.10 (±0.12)	16.1 (±1.83)	2.06 (±0.07)	0.313 (±0.034)	0.70 (±0.085)	1.04 (±0.119)
(3)+(4) Reticular and mixed nodulation plus confluent fibrosis.	19	36	6	1.15 (±0.08)	14.7 (±1.11)	2.17 (±0.15)	0.316 (±0.024)	0.74 (±0.053)	1.11 (±0.085)

\* Years exposure :—1 and 2, years occupied ; 3, years between removal from dust and death.

† (±....) = standard error of the mean.

‡ Figures represent percentages by weight of the dried lung.



TABLE VIA  
*Difference in Mineral Content of Pathological Groups (Anthracite Colliers)*

<i>Contrast.</i>	<i>Total Silica.</i> (Differences between means, with their standard errors ( $\pm \dots$ )†, and t tests, i.e., difference between means $\div$ standard error of the difference, expressed as probabilities, p, that the differences between means are due to chance.)	<i>Coal.</i>	<i>Quartz.</i>	<i>Mica.</i>	<i>Kaolin.</i>
<i>* Mixed nodulation minus reticulation, i.e. (3)–(2)</i>	0.284 ( $\pm 0.149$ ) <u>p=0.10–0.05</u>	0.48 ( $\pm 2.42$ ) >0.5	0.075 ( $\pm 0.047$ ) 0.5–0.1	0.21 ( $\pm 0.097$ ) 0.05–0.02	0.25 ( $\pm 0.157$ ) 0.5–0.1
<i>Confluent fibrosis minus reticulation, i.e. (4)–(2)</i>	0.148 ( $\pm 0.168$ ) p=0.5–0.1	3.28 ( $\pm 2.83$ ) 0.5–0.1	0.067 ( $\pm 0.048$ ) 0.5–0.1	0.12 ( $\pm 0.113$ ) 0.5–0.1	0.06 ( $\pm 0.167$ ) >0.5
<i>Combined confluent fibrosis and mixed nodulation minus reticulation, i.e. (3+4)–(2).</i>	0.200 ( $\pm 0.147$ ) p=0.5–0.1	1.92 ( $\pm 2.39$ ) 0.5–0.1	0.070 ( $\pm 0.042$ ) 0.5–0.1	0.16 ( $\pm 0.098$ ) 0.5–0.1	0.12 ( $\pm 0.148$ ) 0.5–0.1

† Standard error of the difference between means =  $\sqrt{\frac{\sigma^2}{n_1} + \frac{\sigma^2}{n_2}}$ , where  $\sigma$  is the standard deviation of all cases in the groups compared, and  $n_1$  and  $n_2$  are the number of cases in each group.

*Note.*—Underlined values are significant by t test.

has not been possible thus far to obtain many lungs from healthy workers, particularly from bituminous mines in South Wales. Analytical data for such lungs are, however, necessary for understanding the mechanism of the pneumokoniosis of coalminers. In particular, our present data offer no explanation as to why the incidence increases so much with increasing rank of the coal. It is to be hoped that future work will remedy this deficiency.

### DISCUSSION

The least severe form of dust disease seen in South Wales coalminers is that called reticulation. It occurs principally among workers in coal, although rock workers appear to go through a reticulation stage. Histologically, it is characterized by the development of a network of reticular fibrils which ensnare and hold the dust; there is no collagen, and the nodular appearance of classical silicosis is absent. Silicotic nodulation in the miners of South Wales, on the other hand, shows the same whorled redundant\* fibrosis of nodular type as is seen in the silicotic lungs of sandblasters, gold miners, etc. This condition develops in comparatively few of the men, and in those whose lungs were studied in this series it has occurred only among the rock workers who breathe a dust which is (a) relatively poorer in coal and richer in siliceous components and in which (b) the quartz percentage is higher than that breathed in other branches of the industry. In reticular and mixed nodulation, the pathological and X-ray pictures are intermediate between those of reticulation and of silicotic nodulation: reticular nodules are simply foci of dust-reticulation which have become exaggerated to the point of appearing nodal; mixed nodules have the silicotic characteristic of redundant collagenous fibrosis, but they are atypical and are modified by the presence of much dust-laden reticular tissue. Confluent fibrosis signifies local or patchy confluence of these several changes, always including reticulation (Belt and Ferris, 1942).

It is, therefore, the case that we have in this series two distinct types of lung change, one, reticulation, being a mild form of fibrosis caused by proliferation of reticular fibrils, and the other, silicotic nodulation, a severe type of fibrosis caused by massive proliferation of collagen fibres; and that there are two further groups which, in terms of their lung changes, occupy positions between these two extremes. If reticulation is a step in the pathological process culminating in silicotic nodulation, then the aetiological agent or agents which are responsible for the nodulation might be expected to be found in lesser concentration in those lungs which have not progressed beyond reticulation; or, if not in lesser concentration, then diluted by other material. Similarly, lungs which show evidence of intermediate pathological change might be expected to show intermediate concentrations. It had been hoped that the number of lungs analyzed in this investigation would be sufficient to settle these points: it is not certain that such is the case. However, it is advisable to consider what data there are, and permissible to speculate on the causes of dust disease, in order to arrive at hypotheses which may be of use in the planning of further investigations.

The gradual increase in the average concentrations of quartz and mica, seen on passing from one pathological category to another, i.e., from the less severe to the more severe, suggests that these components of the inhaled dust are probably the noxious agents. But the differences between their concentrations in reticulation and in mixed nodulation and confluent fibrosis do not stand the test of statistical analysis, which indicates either that the differences are too small to settle a question of aetiology, or if not too small,

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\* The term "redundant" is used to imply that the amount of fibrous tissue produced in the lesion is greatly in excess of what appears necessary to incorporate the dust (cf. Belt, Ferris and King, 1940).

that there are too few cases to prove it. When we come to silicotic nodulation, however, the increases in concentration have become sufficiently large to indicate that they are probably genuine. It is, therefore, only at the extremes of the pathological picture that the differences in concentration of quartz and mica can be viewed with confidence.

If our supposition is correct, that it is among substances showing real differences that one should look for agents of aetiological significance, then quartz and mica, of the substances investigated, would seem to be the components of the South Wales dust which are primarily responsible for the pneumokoniosis. As pointed out in the introduction, quartz is known to produce silicotic nodulation; and this is probably preceded by a pathological stage in which reticulation is the most prominent feature of the dust-ridden lung (Belt and Ferris, 1942). On the other hand, Jones (1933) demonstrated the presence of micaceous material in rocks and lung residues from South Wales and advanced the theory that this mica (sericite) was the most important causative factor in the lung disease seen in South Wales. The "fine fractions" of the powdered clod strata prepared by Hicks and Nagelschmidt (1943), and shown by them to be almost pure mica, have failed to produce lung changes in animals which are in any way comparable to those produced by quartz (Belt and King, pp. 29-68 below). This failure might conceivably be due to inactivation of the mica through hydration, since in the method of isolation large amounts of water were used. In future work, similar mica fractions should be produced by air elutriation or by sedimentation in a non-polar liquid. If such mica in animal experiments proves as harmless as the fine fractions tested so far it would appear that quartz is the most important aetiological factor of pneumokoniosis in the South Wales dust.

The possibility of coal dust itself—i.e., the coal substance independent of any siliceous minerals it contains—being a factor in producing pneumokoniosis has interested Badham and Taylor (1936); and Gough (1940) has shown that long exposure to coal dust containing very little silica may induce severe lung changes in coal trimmers. The evidence presented in this report has some bearing on the subject. In the first place, there is no evidence that coal can have a part in the production of silicotic nodulation, since its concentration in the lungs showing this change is less than that of any other component, and silicosis can be produced in its absence. Secondly, the average concentrations of coal in reticulation and in mixed nodulation are almost identical, both in the whole series and amongst the anthracite miners. It is true that confluent fibrosis has somewhat more, but the differences lack significance; and there is no other category having definitely more coal than confluent fibrosis, as silicotic nodulation has more quartz and mica. Trimmers might be thought to provide the typical case of a pneumokoniosis developing from coal. These men load the coal into ships and are exposed to very little rock-dust. But even the coal itself—so-called "pure coal"—contains siliceous matter (Brammall and Leech, 1943; Hicks and Nagelschmidt, 1943), and the few lungs of the trimmers (and screeners) studied here have had abnormal concentrations of silica, some of it quartz; and there is no more coal in their lungs than in those of colliers.

In the foregoing discussion, we have stressed the importance of quartz in the aetiology of the South Wales pneumokoniosis. But in doing this, we do not wish to dismiss the other constituents of the dust as being without significance. Haldane (1935) has pointed out that the lung cannot become a "physiological dust trap", and yet retain its normal structure and function; and it would seem probable that heroic quantities of any dust, no matter how innocuous they might be in small concentration, would in time overwhelm the defences of the lung and accumulate in such amount as to impair function. Such a form of lung disease would obviously be due to causes of a mechanical

nature, i.e., the physical presence of large amounts of inert foreign material. Silicosis is not of this variety: it is caused by relatively small quantities of dust, is probably due to a toxic chemical agent, i.e., silicic acid, and the lung changes are out of all proportion to the amount of dust present. But it is possible that reticulation may be of this mechanical type of pneumokoniosis: the fibrous response of the tissue is not out of proportion to the amount of dust, there is only enough reticulin formed to hold the particles in a loose sort of enmeshment, and the redundant fibrosis seen in silicosis is absent. On this reasoning, any type of dust which is non-toxic in the chemical sense (i.e., which does not release a poisonous substance from its surface) might be expected to produce reticulation if it were breathed in sufficient quantity and for long enough. Of the substances studied in this investigation, coal is probably the most inert: no toxic substance is known to be liberated from it. Whether or not dust reticulation could be produced by prolonged exposure to clean coal dust, in the complete absence of siliceous material, cannot as yet be stated; but animals whose lungs have been insufflated with massive doses of clean coal, containing less than 1 per cent. of siliceous matter, have developed only minimal reticulin fibrosis, and the condition is not dissimilar from that produced by some of the mine dusts containing considerable proportions of silicate material (Belt and King, p. 38 below). Moreover, Lochtkemper (1936) described röntgenological evidence of changes in the lungs of soot workers, exposed for many years to massive amounts of almost pure carbon dust, which although not disabling were certainly indicative of a considerable amount of fibrosis, and which appear to have been very similar to the X-ray picture of reticulation described by Hart and Aslett (1942). It is relevant to recall, also, that Schnurer and Haythorn (1937) found that rabbits and rats developed fibrous reactions about the carbon deposits in their lungs with the formation of collagen strands, several months to a year after being subjected to bituminous smoke.

Kaolin and mica liberate to body fluids only a small fraction of the silicic acid which quartz will liberate, and on the chemical theory of silicosis they would appear less likely to be aetiological agents. This supposition is supported by animal experiments. These substances might be supposed to act either as mechanical agents or in a mild chemical fashion like very coarse quartz (e.g., of  $10\mu$  and over), which liberates much less silicic acid per unit weight from its surface than more finely divided respirable material, mostly under  $2\mu$ . This sort of action might produce nothing more advanced than reticulation; while quartz of ordinary respirable size produces silicotic nodulation. But it has already been remarked that silicotic nodulation goes through a reticulation stage. This may be the initial mechanical effect of the quartz particles, or it may be the first evidence of the chemical effect. If the former, then redundant nodular fibrosis is an additional process, which may be superimposed on reticulation, but which does not arise out of it; if the latter, then reticulation is merely a stage in the silicotic process, which may stop short of redundant fibrosis because the chemical stimulus is not sufficiently strong. Most silicates, and mixtures of small amounts of quartz with large amounts of silicates, may act in this way.

These remarks have been directed at the extremes of the pathological picture. What can be said of mixed nodulation and confluent fibrosis? In both these conditions the reticulation element is far more prominent than it is in silicotic nodulation; and this, we suggest, is because of the presence of much larger amounts of diluent dust, such as coal, kaolin and mica, than are found in simple silicosis. These dusts may act in two ways—as simple diluents of the quartz, and as causative agents of reticulation. In the former case, they should diffuse and retard the development of silicotic tissue, and in the latter, they should provide a background of reticular tissue on which the silicotic nodules

would develop. This conception might well account for the distorted and atypical nodules of mixed nodulation, and for the confused coalescent appearance of confluent fibrosis.

All instances of dust reticulation show the presence of abnormal amounts of quartz—from as low as 0.06 per cent. to as high as 0.61 per cent. in the dried lung; and it may reasonably be asked why a silicotic element is not evident in the fibrotic process in the lungs in these subjects. Questions of personal idiosyncrasy aside, two explanations appear possible: the quartz may be so diluted by accompanying dust as to become insufficiently active to produce its characteristic effects; and the silicate minerals present may have rendered the quartz relatively innocuous by their depressant action on “silica-solubility”, whereby the particles of quartz become coated with a layer of alumina and fail to release as much silicic acid from their surface, as they would in the absence of much silicate (see King, pp. 69–98 below). This last point applies to the lungs showing mixed nodulation and confluent fibrosis as well, but their average quartz concentration is somewhat higher than in reticulation, and this, as has already been pointed out, is the only substance investigated in these lungs which can reasonably be associated with the development of silicotic nodulation.

The considerations here outlined, then, lead us to two alternative hypotheses regarding reticulation: that it may be due to any insoluble dust which remains chronically in the lungs in high concentration, or that it may be a sort of “attenuated silica” effect, caused by siliceous dusts, or mixtures of dusts, which liberate only small amounts of silicic acid from their surfaces. The evidence at present available does not make it possible to decide between these hypotheses. Only those dusts capable of liberating silicic acid in sufficient concentration, such as quartz and flint, will produce silicotic nodulation. When men are exposed to mixed dusts of variable composition, such as that breathed in the mining industry of South Wales, either pathological condition may be found, or combinations of the two conditions, or distortions of one by the other.

### SUMMARY

The lungs of 54 workers from the South Wales coalfields have been analyzed chemically for silica, alumina and coal. The minerals contained in the lungs have been isolated as residues, and in these the quartz, mica and kaolin have been determined by X-ray diffraction pattern analysis.

The amount of coal, and the relative proportions of siliceous minerals, found in the lungs reflect the nature of the employment in which the worker had engaged, e.g., collier, hard-header, etc.

The average dust in anthracite colliers' lungs is almost identical in composition with the average air-borne dust at the coalface, and there is no evidence of any change in mineral composition within the lungs.

No relation was found between the severity of the pathological lesions in the lungs and the concentration of either coal or kaolin. The concentrations of mica and quartz, on the other hand, ran more or less parallel with the degree of fibrosis observed, if all workers are considered as a group, i.e., including occupational differences. This correlation, however, is weak and statistically not significant if the 27 lungs of anthracite colliers alone are compared.

Reasons are advanced for dismissing mica and accepting quartz as the major aetiological agent in the production of the South Wales pneumokoniosis. The rôle of the other constituents as diluents or quartz inhibitors is discussed.

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## CHRONIC PULMONARY DISEASE IN SOUTH WALES COALMINERS.—III. EXPERIMENTAL STUDIES

### C.—THE ESTIMATION OF COAL AND OF ALUMINIUM IN DRIED LUNG

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### INTRODUCTION

In the course of work on the pneumokoniosis of coalminers, it was desired to obtain quantitative data on the amounts of coal and aluminium present in the lungs (see King and Nagelschmidt, p. 1 above). An investigation was therefore undertaken of possible methods of estimation.

Badham and Taylor (1936) describe a method for coal in which the lung tissue is destroyed by 25 per cent. sodium hydroxide; the residue is isolated by collecting it adherent to a layer of fat at the top of a separating funnel and is then washed by centrifuging with alcoholic solutions; and the coal is finally estimated by the loss in weight on ashing. Results given by this method were thought to be too high, due to incomplete destruction of the lung tissue in the NaOH digestion. Attempts were made to evolve a method in which the residue from the NaOH digestion was treated with  $H_2O_2$  on the water bath, to destroy the remaining lung tissue. This procedure was found to be unreliable, owing to the action of  $H_2O_2$  on the coal itself. For the same reason, the  $H_2O_2$  digestion procedure of Nagelschmidt and King (1941) was found to be unsuitable for the isolation of coal, although it is effective for isolating siliceous minerals.

An attempt to find an oxidizing agent which could be used with safety was unsuccessful. Potassium permanganate either had little effect on the dry lung, or was too drastic an agent, according to the conditions in which it was used. Bromine water proved to have almost no effect either upon the coal or upon the lung residue. Modifications of the soda digestion were therefore investigated; the effects of larger amounts of soda, longer digestions and multiple digestions were tried. The method finally adopted involves two soda digestions, and the residue is separated by centrifuging, instead of by the less reliable separating funnel technique. Results obtained by this method are constantly lower than those given by Badham and Taylor's single soda digestion; further treatment with soda does not greatly affect the result.

Several methods for the estimation of aluminium were investigated, and a modification of Berg's (1935) 8-hydroxyquinoline procedure was adopted.

### EXPERIMENTS

#### (i) BADHAM AND TAYLOR METHOD (Single NaOH Treatment) FOR COAL

This method was found in preliminary investigations to give good duplicates, but these were undoubtedly due to the similarity of conditions in estimations conducted simultaneously; they may not represent true values for coal. Samples of duplicates are given below. Results are expressed as percentages

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of coal in the various lungs: (1) 17.2; 17.0 (2) 9.2; 9.0 (3) 23.7; 23.9 (4) 11.6; 11.8 (5) 2.67; 2.71 (6) 16.0; 15.8.

In the first stage of their method, Badham and Taylor boil gently a weighed sample (about 2 g.) of dried lung with 30 ml. of 25 per cent. NaOH "until the lung tissue is destroyed. This generally takes about one hour." Actually, the coalminer's lung gives a completely black suspension in soda, and it is impossible at this stage to know if lung tissue has all been destroyed. Microscopic examination of the finely powdered lung material is not very helpful. An arbitrary time must therefore be adopted; in our case 1½ hr. was chosen.

In the next stage of the method, the alkaline solution is made acid with HNO<sub>3</sub>, boiled till yellow-brown (5 min.), transferred while hot to a separating funnel, shaken (with added oleic acid if necessary), and the coal allowed to rise to the top. The liquid remaining below is a light brown colour but contains obvious particles in suspension. These are not black, and presumably are not coal; they look suspiciously like small bits of undestroyed lung tissue. These particles are run off and discarded, the top black layer only being retained; some of the tissue (?) particles, however, have adhered to the oil and are collected with the coal.

Longer soda digestions were tried, but it was found that up to 6 hrs. particles still remained in the liquid. The digestion is difficult beyond 2 hrs., owing to a tendency to bumping of the concentrated soda and amino acid mixture.

## (2) HYDROGEN PEROXIDE DIGESTION

At this stage, various attempts were made to destroy the particles (? residue of lung tissue) remaining after Badham and Taylor's soda digestion, by treatment with H<sub>2</sub>O<sub>2</sub> on the water bath. Ninety and 100 volume H<sub>2</sub>O<sub>2</sub> solutions were used, in varying amounts; and different times of digestion were tried. A preliminary trial with a single digestion of anthracite coal dust as a control showed no significant effect of peroxide; bituminous coal, however, showed a notable loss of weight on peroxide digestion.

The residue left by the Badham and Taylor soda treatment (after washing) was given multiple peroxide treatments, being dried and weighed after each. Only lungs containing anthracite coal were used. The results, given in Table VII, show a continuous fall in weight of the residue. The specimens treated the longest were greyish-white when the treatments were discontinued. It was obvious that the coal was being attacked.

TABLE VII

*Effect of H<sub>2</sub>O<sub>2</sub> on Lung Residues after NaOH Treatment (showing destruction of coal by continued H<sub>2</sub>O<sub>2</sub> treatment)*

Lung No.	Amounts of H <sub>2</sub> O <sub>2</sub> (ml.).	Duration of heating (hours).	Weight of residue (g.)		Weight of residue as percentage of original lung tissue.	
			(1)*	(2)*	(1)*	(2)*
P.154 .. ..	0	0	0.2326	0.2661	11.6	13.0
	10	4	0.1512	0.1881	7.5	9.2
	10	4	0.0985	0.0968	4.9	4.7
P.154 .. ..	0	0	0.2462	0.2591	12.2	12.4
	10	5.	0.1004	0.1073	5.0	5.1
	5	5	0.0485	0.0601	2.4	2.9
P.174 .. ..	0	0	0.3656	0.3590	18.2	17.7
	6	3	0.2667	0.2531	13.3	12.4
P.185 .. ..	0	0	0.6020	0.6024	30.0	30.0
	10	3	0.5782	0.4614	28.6	23.0
P.154+added charcoal.	0	0	0.5989	—	25.3	—
	10	3	0.5538	—	23.5	—

\* (1) and (2), duplicate experiments.



Multiple peroxide treatments were then tried on anthracite and on bituminous coal. The results are given in Table VIII. Both types of coal lose weight on prolonged treatment with  $H_2O_2$ , after an initial period of resistance. Anthracite coal appears to be more resistant than bituminous coal, and loses weight more slowly. To all appearances, the behaviour of pure coal, when treated with  $H_2O_2$ , differs from its behaviour in a lung residue; this, however, may possibly be an artefact; in the lung residue the first loss of weight is probably due to destruction of the remaining lung tissue, and later loss to action of the reagent on the coal.

Hydrogen peroxide was thus shown to be an unsuitable reagent for use in the determination of coal.

TABLE VIII  
Effect of  $H_2O_2$  on Coals

Type of coal.	Direct $H_2O_2$ treatments.		Weight of coal as percentage of its original weight.	
	Amounts of $H_2O_2$ (ml.).	Duration of heating (hours).		
Anthracite A3 .. ..	0	0	100.0	
	20	5	100.5	
Bituminous B1 .. ..	0	0	100.0	
	20	6	97.0	
	10	3	43.2	
Anthracite A2 .. ..	0	0	100.0	
	10	3	101.3	
	15	4	100.7	
Bituminous B2 .. ..	0	0	100.0	
	10	3	107.6	
	15	4	77.1	
<i>H<sub>2</sub>O<sub>2</sub> after NaOH treatment.</i>				
Anthracite A2 .. ..	0	0	100.0	100.0
	10	4	98.3	97.3
Anthracite A3 .. ..	0	0	100.0	100.0
	15	4	105.0	106.0
	20	5	84.8	96.2
	20	4	73.6	55.8
	15	3	47.2	39.3
	10	3	28.2	27.3

(3) MULTIPLE SODA TREATMENTS

It is reasonable to suppose that the tissue of a silicotic human lung, often extensively fibrotic, should be considerably more resistant to the action of concentrated soda than, for example, liver tissue, which is so successfully destroyed by alkaline digestion in Pflüger's method for glycogen estimation, or than animal lungs, which gave the following results after a single digestion with NaOH :-

Percentage of "Coal" in Dried Lung.

Horse .. .. .	1.46; 1.04
Cow .. .. .	1.07

These residues were brown in colour (not black), and the figures must be considered to represent residual lung tissue, and not coal. With fibrous tissue,

the destruction is probably still less complete. It was considered, therefore, that larger amounts of soda and longer digestions might be necessary. The possibility that accumulation of the products of digestion might tend to slow down the hydrolysis suggested also that treatment with fresh soda might be advisable.

To test these possibilities, a number of lung tissues were given multiple soda treatments, varying amounts and times being used, and the residues were weighed after each treatment. It was found more practical, as well as undoubtedly being more nearly quantitative, to separate the coal from the alkaline solution after digestion, by centrifuging in about 50 per cent. alcohol. Badham and Taylor's alcoholic KOH and acid alcohol were used for washing.\* The method tried was as follows:

About 2 g. of dried lung were weighed accurately and placed in a beaker of suitable size. Soda was added (30 to 250 ml.) and the mixture boiled slowly for the desired length of time ( $1\frac{1}{2}$  to 4 hr.), distilled water being added from time to time as the mixture concentrated. The mixture was then transferred to a 100 ml. centrifuge tube, washed in with a very little water, and a volume of 95 per cent. or absolute alcohol equal to the amount of soda used was added. (With the larger amounts of soda, it was necessary to add the alcohol in the beaker and centrifuge in 100 ml. lots.) The mixture was thoroughly stirred and well centrifuged, decanted, washed twice with 20 ml. hot alcoholic KOH, and three times with 20 ml. acid alcohol. The residue was then transferred to a weighed 100 ml. pyrex beaker by means of small amounts of water; dried to constant weight, and weighed. For a second treatment, soda was added to the residue and the process repeated.

The results on different lungs are recorded in Table IX. It will be seen that in every case there was an appreciable loss of weight in the second soda treatment, indicating that the first treatment, even when quite large amounts of soda and up to 4 hrs. digestion were used, was insufficient. The amount of loss of weight did not correspond to the amount of coal present, nor did it bear a relation to the amount of soda used; it is probable that lungs of different degrees of fibrosis vary in the ease with which they are attacked by soda.

It is obvious that an appreciable proportion of the residual lung tissue is destroyed by the second treatment with fresh soda. A slight loss of weight is shown after the third soda digestion, but its magnitude is much less. A fourth treatment produces a further very slight fall in weight, comparable to the experimental error (see data for anthracite coal below). Thus a good approximation to the true coal value may be obtained by two or three soda digestions.

The effect of multiple soda digestions on samples of clean coal is shown in Table IXA. The anthracite sample remained at a comparatively steady level, and its fluctuations probably represent the degree of error likely to arise in the method. The bituminous coal does, however, show a slight loss of weight due to the soda treatment, the loss being greatest in the first treatment. Results on lungs containing bituminous coal cannot, therefore, be as accurate as results on anthracite lungs.

#### (4) METHOD ADOPTED FOR THE ESTIMATION OF COAL

The method finally adopted represents a compromise between practicability and accuracy. Two soda digestions are employed, thus eliminating the greater part of the inaccuracy of Badham and Taylor's technique. The use of three digestions was rejected, as making the method too long and tedious without

\* In a few cases, the alkaline digest was acidified and filtered, being washed by decantation. Suction filtration was not possible, and gravity filtration and washing took many hours. Centrifugation is quicker and was generally employed.

TABLE IX  
Effect of Multiple NaOH Treatments on Dried Lungs

Lung No.	Residue (as percentage of lung) remaining after				Amounts of NaOH (ml.).				Time digested (hours).			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
	NaOH treatment.											
A ..	7.6	5.5	5.3	5.2	150	50	50	50	1½	1½	1½	1½
B ..	11.5	10.3	10.0	9.8	150	50	50	50	2½	1½	1½	1½
C ..	2.13	1.99	1.85	—	250	50	50	—	4	1½	1½	—
P.154 ..	16.9	14.0	—	—	200	50	—	—	2½	1½	—	—
P.175 ..	29.5	27.8	27.3	25.8	100	50	50	50	1½	1½	1½	1½
	29.7	27.8	27.3	27.0	100	50	50	50	1½	1½	1½	1½
P.115 ..	16.3	15.7	15.2	15.0	30	50	50	50	1½	1½	1½	1½
	16.3	15.6	15.2	15.0	30	50	50	50	1½	1½	1½	1½
P.121	8.1	6.8	6.2	5.9	50	50	50	50	1½	1½	1½	1½
	8.0	6.8	6.2	5.9	50	50	50	50	1½	1½	1½	1½
P.145 ..	8.3	7.8	7.7	—	100	50	50	—	1½	1½	1½	—
	8.4	7.8	7.6	—	100	50	50	—	1½	1½	1½	—

COAL AND ALUMINIUM IN DRIED LUNG

TABLE IXA  
*Effect of Multiple NaOH Treatments on Clean Coals*

Coal sample.	Percentage of sample remaining				Amounts of NaOH (ml.).			Time of digestion (hours).		
	before	after								
		NaOH treatment.				1st	2nd	3rd	1st	2nd
		1st	2nd	3rd						
Anthracite ..	100.0	99.5	100.8	100.1	50	50	50	1½	1½	1½
	100.0	99.6	100.9	100.3	50	50	50	1½	1½	1½
Bituminous ..	100.0	97.1	95.3	93.7	50	50	50	1½	1½	1½
	100.0	96.2	94.2	92.6	50	50	50	1½	1½	1½

any very great increase in accuracy. The use of centrifugation in alcohol is an improvement on the separating funnel separation; 40–45 per cent. alcohol was found to be satisfactory. The method as used is moderately accurate, probably within  $\pm 5$  per cent. for anthracite lungs, while for bituminous lungs the extra error due to soda action on the coal will tend to cancel the primary error due to undestroyed lung tissue, and the accuracy is difficult to assess. The method gives consistently good duplicates. Examples were as follows, the figures representing percentages of coal in dried lung tissue:—(1) 13.8; 13.9 (2) 9.7; 9.5 (3) 26.4; 26.7 (4) 12.8; 12.8 (5) 1.9; 1.9 (6) 16.0; 16.1.

*Procedure.*—About 2 g. of dried lung tissue are weighed accurately and transferred to a 250 ml. pyrex beaker. 100 ml. of 25 per cent. NaOH are added and the mixture is boiled very gently for  $1\frac{1}{2}$  hours, with a watch glass covering the beaker. It is then partially cooled, and 80 to 90 ml. alcohol are added slowly, with constant stirring. When well mixed, the suspension is poured into a 200 ml. centrifuge tube (or in two lots into 100 ml. tubes) and centrifuged until the supernatant is free from coal. The supernatant is then discarded, and the residue returned to the original beaker by means of small quantities of 25 per cent. soda. The volume is made up to 50 ml. with more soda, and the mixture is again digested slowly for  $1\frac{1}{2}$  hours.

After cooling slightly, the mixture is poured into the original centrifuge tube, and all particles washed in with small amounts of water. 40 ml. alcohol are added, the mixture stirred thoroughly and centrifuged as before. The supernatant is discarded, and the residue washed twice with hot alcoholic KOH and three times with acid alcohol. It is then transferred to a weighed platinum dish by means of small amounts of water, evaporated to dryness, dried to constant weight at  $110^{\circ}$  C., and ashed at dull red heat. The loss of weight on ashing is taken as a measure of the coal present.

#### Reagents

- (1) 25 per cent. NaOH
- (2) absolute or 95 per cent. alcohol
- (3) alcoholic KOH (20 g. KOH, 300 ml. water, 600 ml. 95 per cent. alcohol)
- (4) acid alcohol (100 ml. conc. HCl, 200 ml. water, 600 ml. 95 per cent. alcohol).

TABLE X  
*Comparison of Badham and Taylor Method with Multiple NaOH Treatment Method*

Lung No.	Percentage of coal in lung.	
	Badham & Taylor method.	Multiple NaOH Treatment.
P.115 .. ..	17.1	14.6
P.121 .. ..	7.7	5.4
P.145 .. ..	9.1	7.3
P.154 .. ..	11.7	9.4
P.174 .. ..	15.9	12.8
P.175 .. ..	30.8	26.0
P.181 .. ..	2.7	1.3
P.185 .. ..	23.8	18.9

A comparison between results given by this method and those given by Badham and Taylor's procedure is recorded in Table X. The consistently higher figures shown by Badham and Taylor's method will be observed. The differences are thought to be due to the presence of undestroyed lung substance in the residues obtained with the Badham and Taylor technique.

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### (5) METHOD ADOPTED FOR THE ESTIMATION OF ALUMINIUM

About 20 mg. of ashed lung or of lung residue are weighed, fused with 100 mg.  $\text{Na}_2\text{CO}_3$ , dissolved in water and neutralized to methyl orange with HCl. 5 ml. of 30 per cent. hexamine are added, the mixture heated to flocculate the  $\text{Fe}(\text{OH})_3$  and  $\text{Al}(\text{OH})_3$ , centrifuged, decanted, and washed three times with hot water. The  $\text{Al}(\text{OH})_3$  is dissolved by heating in 5 ml. N/2 NaOH, and centrifuged. It is sometimes difficult to dissolve completely and further extractions with 5 ml. N/2 NaOH are necessary. Each extract and washings are diluted to about 60 ml. and treated with 0.5 ml. glacial acetic acid. The solutions are heated to  $50^\circ$ , 10 ml. of 1 per cent. 8-hydroxyquinoline\* are added to each, followed by 10 ml. of saturated sodium acetate solution. They are heated for a further 5 minutes. All solutions are filtered hot through the same weighed sintered glass filter prepared with a layer of asbestos, washed with hot and then with cold water, dried at  $100^\circ$  and weighed. The precipitate obtained contains 5.87 per cent. Al.

Using about 20 mg. of the ashed and acid extracted lung residue described by King and Nagelschmidt (p. 3), a precipitate of aluminium hydroxyquinoline weighing 40 to 60 mg. was obtained from most of the South Wales lungs examined. Duplicate samples were in good agreement, as shown by the examples below:—

No.					Per cent. aluminium.	
1	..	..	..	..	25.4	26.0
2	..	..	..	..	24.7	24.5
3	..	..	..	..	27.0	27.3
4	..	..	..	..	26.3	26.9

\* 1 g. hydroxyquinoline is ground with 1 ml. glacial acetic acid and dissolved in 100 ml. hot water.

### SUMMARY

- (1) A method for the estimation of coal is described.
- (2) Its accuracy is considered to be generally within  $\pm 5$  per cent.
- (3) Defects in other methods are discussed.
- (4) An 8-hydroxyquinoline method for aluminium is described.

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# CHRONIC PULMONARY DISEASE IN SOUTH WALES COALMINERS.—III. EXPERIMENTAL STUDIES

## D.—TISSUE REACTIONS PRODUCED EXPERIMENTALLY BY SELECTED DUSTS FROM SOUTH WALES COALMINES

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### 1. OBJECT OF RESEARCH

As a part of the investigation of chronic pulmonary disease in South Wales coalminers, it was determined to study a variety of dust samples from the mines with a view to assessing their pathogenic effects upon animals. By this means, it was hoped to identify the more noxious minerals to which the miners are exposed.

That the pneumokoniosis of coalminers is produced by a mixture of dusts has been shown in *Medical Research Council Special Report Series No. 243, 1942*. In any given mine the collier inevitably inhales particulate matter derived from various geological strata through which the workings must proceed in order to win the coal. He inhales the dust of various stony strata, as well as of the coal itself. The experiments here recorded were therefore planned with the idea of attempting to assess the relative pathogenicity of each of these dusts, in so far as they could be isolated and tested as separate components of the dust hazard as a whole.

### 2. SCOPE OF EXPERIMENTS

To begin with, a high incidence\* anthracite mine was chosen. The various strata of this mine are diagrammatically represented in Fig. 1, facing p. 68. Samples of each stratum were carefully collected and prepared† for experimental application to animals. Certain air-borne dusts were also obtained from the same mine.

Only the one mine was systematically investigated, stratum by stratum, but from other mines of low pneumokoniosis incidence as well as high—bituminous as well as anthracite—various representative dusts were included in the scheme, for the sake of obtaining a wider range from which to draw comparisons. All told, 41 individual samples as well as 14 combinations (e.g. quartz plus coal) were investigated.

### 3. EXPERIMENTAL PROCEDURE

How best to assess the pathogenicity of such a wide variety of dusts was a problem not easily resolved. It was necessary to adopt some standard procedure, so as to maintain a uniform basis for comparison of results. After considering various alternatives such as subcutaneous injection, the inhalation (dusting chamber) method of Gardner and the intraperitoneal technique of Miller and Sayers (1934), it was finally decided to employ the intratracheal method of Kettle and Hilton (1932).

*Technique.*—Twelve animals were used for each dust sample. A single dose of known quantity was administered in fluid suspension to each animal, by injection from a hypodermic syringe directly into the trachea. Each animal was anaesthetized, and, with aseptic precautions, the trachea was exposed, the dust injected and the incision closed by suture.

*Animals.*—The rat proved to be the most satisfactory animal for the tests, and was used almost exclusively. The white or black and white variety of tame or "gentled" rat served very well. They are hardier and more economical than guinea-pigs. They average about 250 g. in weight when fully grown, and their normal span of life in the laboratory is about 2 years. Counting the experiments which had to be repeated, close on to 1,200 rats were used.

*Preparation of inoculum.*—Each dust had to be evenly suspended in a fluid medium, so that it would flow smoothly and quickly from a syringe fitted with a relatively fine needle (gauge 12). In many cases, a satisfactory suspension was obtained by simply shaking the dust in saline. Though inclined to settle out rather quickly, the particles could be held

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\*High incidence of pneumokoniosis among the miners.

† Large, hand-picked samples of each stratum were supplied by Major D. Jeffreys. These were reduced to a fine powder in a rubber-lined tumble-mill. Chemical analysis by one of us (E. J. K.) and petrographic analysis by Dr. A. Brammall showed that the dusts so prepared were not significantly different in composition from the gross samples.



well enough in suspension by gently shaking the inoculum right up to the moment of injection. Once the needle entered the trachea, the inoculum was expelled rapidly and with some force, so as to drive it into the lungs under pressure and thus obtain as much dispersal as possible. Some of the dusts, however, especially the coaly samples, were not so easily prepared for test. They tended to flocculate in saline, and could not be suspended adequately except by the addition of an emulsifying agent. This, of course, presented certain problems, for the emulsifying agent had to be of such a character as not to complicate the experimental results. Fortunately, it was found possible to overcome the difficulty by using small quantities of cow's milk. The milk was skimmed, boiled and the clot removed, and it was then mixed with equal quantities of saline. Even the most refractory dusts, when shaken up in this fluid were, with but few exceptions, satisfactorily suspended. Suitable control experiments were performed to see if the milk itself, so treated, produced any significant histological change in the rat lung or modified the tissue reaction to dust particles; the results were negative.

The saline or milk-saline was always sterilized, and in most cases the inoculum was autoclaved before use. Certain dusts, however, were injected "raw", to see whether soil bacteria and other organisms inherent in the dust as breathed by the miners tended to modify the histological reaction.

*Dosage of dust.*—A healthy rat will tolerate quite well 1.5 to 2 ml. of fluid insufflated into the trachea, and will retain most of it, though small quantities sometimes escape from the nostrils. Since a quantity larger than 2 ml. is likely to drown the animal, the dose of dust must be conveyed in a relatively small volume of fluid. This restriction has proved to be quite compatible with an adequate dosage of practically all the dusts with which we had to deal. Finely ground stone or clay, for example, will suspend satisfactorily in dilutions of 150 mg. per ml., and 200 mg. of such dusts is more than an adequate dose. With coal, the amount has to be more limited, but 60 to 100 mg. is not difficult to manage, and a dose of this magnitude, which is sufficient to stain the lungs black, was invariably employed; 100 mg. of dust would constitute about 25 per cent. of the dry weight of a rat lung.

*Animal mortality.*—With simple ether anaesthesia for the injections, and the dosage restricted as indicated above, the initial mortality is practically nil, except in tests of certain bulky coal dusts of relatively low specific gravity. Because the inoculum is rather thick, the latter dusts tend to obstruct the bronchioles and to give rise to collapse of the lungs with unfavourable sequelae. In the few experiments where trouble of this sort was encountered, we found that the heaviest mortality occurred within 24 hours. Animals dying within this interval were discarded and replaced. A successful injection was arbitrarily defined as one in which the animal survived at least 24 hours, and each experiment called for at least 12 successfully injected animals. There was but a low mortality rate (under 2 per cent.) from pneumonia within the first week. Thereafter, the animals appeared to be healthy, but their expectancy of life was less by from 3 to 6 months on the average, than that of normal controls.

*Duration of experiments.*—The work was planned in terms of long-range or "survival" experiments, since the primary object lay in assessing chronic rather than acute reactions. It was hoped to obtain lesions of comparable chronicity to those which characterize the pneumokoniosis of coalminers. Consequently, most of the animals were allowed to survive for as long as was practicable. A duration of at least 12 months was aimed at in all the experiments, and in the majority this was achieved. No animals survived longer than 18 months. Some experiments had to be terminated under one year, though none under 8 months.

Though end-stage results were our chief concern, two animals in each experiment were killed between the 3rd and 6th days, so that the initial reaction could be noted. Others died or were killed at intervals, thus providing an opportunity to study the progress of the lesions.

From start to finish, the experiments extended over a period of three and a half years.

#### 4. BASIS OF ASSESSING EXPERIMENTAL RESULTS

The dust effect in the animal lung may be judged on the basis of a few simple criteria, namely (1) initial inflammatory reaction, (2) effective retention of dust, (3) connective tissue reaction, (4) density of reticulin fibrosis, and (5) distribution of lesions.

(1) Excluding complicating factors, *initial inflammation* is indicative of an immediate irritant action on the part of the dust. It is important also as the determinant of the type of organization to follow, and therefore has a considerable influence on the end result. Except in a few cases, probably complicated by infection, the initial reaction showed no great fluctuations

in intensity. In the animals surviving the initial reaction (and this includes the great majority), we have no accurate means of assessing the extent or severity of inflammation. Consequently, in the tabulated results (Tables XI to XX), it is simply noted as present or absent in the two or three animals of each experiment examined between the 3rd and 6th days.

(2) *Effective retention* is defined arbitrarily as permanent storage of dust in the fixed tissues of the lung, in contradistinction to casual retention, in which the dust is lodged in potentially mobile, loose-lying cells without engaging the fixed tissues. Effective retention is brought about only when dust deposits are overrun and secured by connective tissue, a process which is conveniently referred to as organization of the dust.

If it could be determined what proportion of each dust was effectively retained and what proportion casually retained, it would assist in gauging relative pathogenicity, but this has proved impracticable, because all but a few samples showed a high degree of effective retention (roughly estimated as from 80 to 100 per cent.) and there is no adequate means of measuring differences within such narrow limits. Perhaps the artificial method of administering the dust in a single massive dose directly into the lung has the effect of obscuring variations in degree of effective retention—variations which would otherwise obtain, under a different method of administration (e.g. by inhalation). Forcing the dust in large quantities virtually into the alveoli favours retention, particularly in the rat lung, where lymphatic drainage is apparently quite unequal to the task of emptying dust-ridden air sacs. Elimination seems to be almost precluded by virtue of the bulky dose; resolution or restoration of the *status quo ante* evidently cannot take place; stagnation results and every opportunity is afforded for organization. In these circumstances, it makes little difference whether the dust is organized or not, for it is more or less permanently retained in either case. Hence the factor of retention is better considered as an index of absolute rather than of relative pathogenicity. The degree of retention (putting aside for the moment the distinction between effective and casual retention) is high and more or less constant for all the dusts under experiment. It becomes, therefore, a quantitative criterion of pathogenicity, and establishes the diagnosis of experimental pneumokoniosis in practically all of the animals. Without this assurance, it might reasonably be asked whether our animals retained enough dust to qualify as bona fide subjects of pneumokoniosis.\*

It is possible to estimate roughly the degree of effective retention in any given experiment by running the eye over the histological sections under very low power magnification and judging the proportion of organized to unorganized dust. This was done, and the estimations arrived at are set down in Tables XI to XX. Observations thus made are not without some value as a guide to relative pathogenicity. A few dusts belonging to groups (c) and (j) were less than 50 per cent. effectively retained after lying for several months in the animals' lungs. These were the least pathogenic in the whole series of dusts (see Tables XIII and XX).

(3) *Connective tissue reaction*. Organization of dust deposits proceeds in one of two ways, or sometimes as a mixture of both:—(a) As a foreign-body reaction, or (b) as an organization of inflammatory exudate hereafter referred to as "*carnification*". According to which of these characterizes the reaction, a dust is classified as of lesser or greater pathogenicity.

(4) *Reticulin fibrosis*, as opposed to collagen fibrosis, measured in terms of reticulin fibrils, constitutes the most important single criterion of pathogenicity. Reticular tissue is the histological basis of coalminers' pneumokoniosis (see

\* The definition of this term used in *M.R.C. Special Report Series No. 243* was: "any dust-ridden state of the lungs sufficient to impair health".

*M.R.C. Special Report Series No. 243*), and it assumes a similarly fundamental role in experimental pneumokoniosis, thereby affording a significant basis of comparison between the two.

Whether in animals or in man, the *density of reticulin reaction* produced by any given mineral dust is the best available histological index of its chronic harmfulness.

It may not be amiss to review briefly the *characters of reticular tissue*.<sup>\*</sup> This is probably derived from the reticulo-endothelial system, and may be regarded as a parent type of connective tissue. It is highly specialized in the function of dealing with foreign particulate matter and, at the same time, multipotential, as shown by the number of ways in which it may differentiate; it may be relatively cellular or acellular, and its fibres may have the property of becoming transformed into collagen.

Whatever the type of organization, newly laid-down reticulin fibrils inevitably make their appearance in the form of net-like snares which tend to secure the dust deposits in continuity with adjacent connective tissue elements. Reticulin fibrils and the cells to which they belong constitute a simple, delicate and inconspicuous form of stroma. It is durable and more or less permanent, like gliosis in the central nervous system. Reticulin, like glial, elastic and collagen fibres, is resistant to cell lysins and undergoes regression by collapse and atrophy, only when it has outlived its function. As the fibres age and mature, they tend to become coarser and tougher. By a slow process of swelling and hyalinization, they lose their affinity for silver stains and tend to be transformed gradually into collagen bundles, but this change is much less evident in experimental than in human pneumokoniosis, probably because of the shorter time-interval involved in the former.

A typical dust-laden meshwork of reticulin is something like a cobweb, but the fibres are laid down without an orderly pattern (see Figs. 3, 25, 26, 28, 29). The *density of the fibrillar mesh* varies greatly with different dusts, and with the age of the lesion. It is precisely this fibrillar density which we sought to determine in the case of each dust sample tested. Unfortunately, it does not lend itself readily to any accurate method of measurement, so we resorted simply to a series of photo-micrographic records, by which to make a rough quantitative comparison of the fibrillar reaction in the various experiments at uniform time-intervals after injection. Care was taken to have the sections of uniform thickness for this purpose.

It seemed advantageous at the outset to establish a *basic reticulin reaction* of standard character, produced by a pure dust of known composition and pathogenicity, with which to compare the less known effects of the various dusts under experiment. The natural choice was, of course, quartz, than which no other mineral is better known as a fibrogenic agent. A typical quartz reaction is therefore included as a kind of "control," chiefly so that the density of its reticulin fibrils may be compared with that found in the other reactions. Density of the fibrillary reticulum ("reticulin fibrosis" in the tabulation of results to follow) in the quartz reaction is maximal in comparison with all other reactions herein recorded, and is arbitrarily designated Grade 4. Minimal fibrosis, as found in the reaction to pure coal, is correspondingly designated Grade 1.

The demonstration of reticulin requires a special technique. Ordinary histological preparations yield no indication of the amount or extent of reticulin present, because the fibrils are not selectively stained by any of the common histological dyes. The fibres are extremely fine, comparable in diameter to *Spirochaeta pallida*, and like the spirochaetes can be differentiated in sections only after silver impregnation. Reticulin is best demonstrated by one or other of the silver techniques. We used Wilder's method exclusively.

Serial sections were always employed in conjunction with the reticulin stain, so that the microscopic fields examined by this method could be studied side by side with neighbouring sections treated in a number of different ways. A series of six consecutive sections of 5 $\mu$  thickness was cut as a routine from each block; No. 1 was stained by haematoxylin and eosin, No. 2 by Wilder's reticulin method, Nos. 3 and 4 were incinerated, No. 5 was stained by Weigert's iron haematoxylin and van Gieson, while No. 6 was kept in reserve for an additional special stain if required. One of the incinerated sections was treated with concentrated HCl to remove everything but the silica residue, which could then be compared precisely with the reticulin topography in slide No. 2.

(5) *Distribution of lesions* provides a further guide to pathogenicity. The less noxious dusts, producing only the simplest of foreign-body reactions, tend to be dispersed into smaller and smaller aggregations, which become more and more widespread as time goes on. This process (which is designated as

<sup>\*</sup> Term used by Maximow and Bloom, *Textbook of Histology* (1931), p. 95, meaning reticulin fibrils in general and their distribution, just as "elastic tissue" is used for the description and distribution of elastic fibres.

"scattering" in the tabulation of results) may be regarded as a form of resolution, by virtue of which the foreign material is fitted into the structure of the lung, presumably in such a way as to produce a minimum of embarrassment to respiratory function. More harmful dusts, on the other hand, tend to retain a nodular distribution, and sometimes coalescence is carried to the point of producing large confluent lesions. Coalescence may be evidence of an inflammatory complication—for example, pneumonia.

Thus, in the tabulated results of individual experiments as well as in the legends of the microphotographs, histological changes are noted in conformity with the foregoing plan, under the following headings: (1) Initial inflammation, (2) Effective retention, (3) Type of organization, (4) Distribution, and (5) Reticulin fibrosis.

## 5. OUTLINE OF RESULTS

Before dealing with the particulars of individual experiments, it will be useful to make a general survey of the results common to all the experiments.

### *Initial Distribution of Dust*

Following the administration of a single massive dose of dust, there is at first a more or less general flooding with dust of the bronchi and bronchioles. Breathing is momentarily suspended, and there is a period of apnoea, lasting as a rule from 20 to 40 seconds. Respiration begins again with a few deep, gasping breaths. Then dyspnoea supervenes, and the flood of inoculum in the air passages quickly ebbs; a minor proportion escapes through the nose, while the rest overflows into the lung. An individual lobule receives a quantity of inoculum roughly corresponding to the volume of its tidal air. In this way, the dust undergoes a lobular distribution (Figs. 5, 8), each lobular unit receiving a roughly similar quantity, which fills perhaps a fourth of its air sacs. Each apportionment of dust is more or less "en masse", occupying a group of contiguous air sacs adjacent to the alveolar duct. In the course of time the distribution tends to alter, as indicated below.

### *Eventual Distribution*

Nodulation is characteristic in the early stages of all experiments. It results from the way in which the dust is distributed, so much to a lobule. The nodules are small, much smaller than those of human pneumokoniosis, belonging to a submiliary order of size. Sometimes, however, they are large enough to fill a lobular unit, and groups of these nodules lying close together may give rise to large composite or confluent lesions.

The nodular dust deposits tend to undergo variable degrees of *scattering* in course of time, as the experiments progress. Some dusts, particularly the coaly varieties, show this tendency more than others, but it may be stated as a general rule that nodulation becomes finer and less distinct the longer the animals survive (see below, under "Progress of Lesions"). At first compact, the dust deposits become thinned out, broken up into smaller aggregations and stretched out unevenly along the alveolar septa (Figs. 12 and 13), simulating the appearance of dust reticulation in the human lung. Notable exceptions to this general rule are to be found in certain instances where, apparently as a result of inflammatory change, coalescence, rather than dispersal, takes place, and large confluent lesions result (Fig. 30).

### *Involvement of Lymph Glands*

A certain amount of dust invariably finds its way into the peribronchial glands (Figs. 23, 29), but only a small proportion of the total dose is thus transported. Lymphatic drainage towards the hilus seems to be more limited in the rodent than in the human lung. Dust deposits in the root glands apparently

reach a maximum early—not infrequently, as early as the third week—and after three months no appreciable increase is observed. Characteristic lesions often make their appearance in the root glands in advance of corresponding lesions in the lung parenchyma; these may serve the useful purpose of yielding quicker results, but we have not been content to rely upon them as truly representative, for they occasionally turned out to be only isolated or perhaps abortive lesions, which, so far as we could tell, were neither accompanied nor followed by corresponding changes in the lung proper.

#### *Progress of Lesions*

The sequence of events may be visualized conveniently under four headings, namely (1) Phagocytosis, (2) Inflammatory change, (3) Organization, and (4) Reticulin fibrosis. Each is characterized by a distinctive morphological feature: phagocytosis by the koniophage, inflammation by fibrin, organization by the fibrocyte or dictyocyte, and fibrosis by reticulin. One important cellular element, the foreign-body giant cell, is often present throughout all four stages.

(1) *Phagocytosis*.—Once lodged in the parenchyma of the lung, all dust particles are quickly phagocytosed. It is remarkable, considering the large amount of foreign material suddenly introduced, that within 24 hours or less practically all the particles are already intracellular. This general “tidying up” is accomplished with extraordinary efficiency and despatch. The principal cell involved in this activity is a large, wandering, amoeboid, mononuclear cell, which we prefer to designate as the koniophage, but which is also known as dust-cell, endothelial cell, macrophage, histiocyte, etc. Particles up to  $10\mu$  in size are dealt with almost entirely by this type of cell. For larger particles, the foreign-body giant cell is usually called into play.

Multinuclear foreign-body giant cells form a conspicuous feature of the reaction to those dusts which contain particles larger than  $10\mu$ , principally coal dusts. Unlike the koniophages, these giant cells tend to remain unaltered throughout the duration of the experiment. In the process of organization, instead of becoming superseded by fixed tissue like the koniophages, they simply become incorporated in it, and retain their identity. Theirs is a peculiarly distinctive appearance but somewhat deceptive to the unpractised eye. Their nuclei are obscured by coal particles, which are peripherally disposed, often in such a way as to form a perfect rosette. One sees a dense black ring surrounding a clear body of cytoplasm. Standing by themselves, they may be mistaken for miniature nodules. The appearance is so uniform and so commonly met with as to merit special attention (Figs. 12 and 17).

(2) *Definite inflammatory change*, usually mild but occasionally intense, was found in approximately 50 per cent. of all animals examined at autopsy within 3 weeks after injection. It showed up most clearly from the 3rd to the 6th days, so this interval was chosen in which to make routine examinations of at least two animals from each experiment.

By the term “definite inflammatory change” we mean the presence of extravasated fibrin demonstrable by Weigert’s stain. Commonly, it is mingled with the dust-laden koniophages in small shreddy deposits and accompanied by a little fluid exudate (which leaves a faint albuminous coagulum in paraffin sections) and occasional granulocytes. It is not the sort of reaction one would ordinarily characterize as a pneumonia. Indeed, it is often so inconspicuous that only the practised eye, aided by special stains and the fore-knowledge of its appearance, would be inclined to accord it adequate recognition. For it is, in fact, a real pneumonia and, for all its apparent mildness, entails sequelae of greater consequence than the ordinary run of more typical pneumonias. In a sense it might be described as catarrhal pneumonia, since the koniophages which make up the bulk of the exudate are, in fact, desquamated cells such as characterize a catarrhal reaction.

It is not always uniformly distributed. Though linked with the dust in distribution, it does not invariably accompany all the dust deposits. It sometimes affects only part of the dust-ridden lung.

It seems fairly clear that the intervention of inflammatory change is not determined solely by the dust-inoculum. Even pure quartz in saline suspension, which yields perhaps the most uniform results of all the dust samples, sometimes shows a patchy exemption from inflammatory change; while some of the coal dusts, which as a rule provoke little or no inflammation, are sometimes accompanied by definite inflammatory changes.

Since this initial inflammation plays an important part in determining subsequent changes, and since it is somewhat unpredictable, not to say capricious, in incidence, it seems essential to outline clearly its variations and possible aetiology.

The quality of the dust has, of course, something to do with it. The stony dust samples are attended by inflammatory change with greater frequency than the coaly dusts.

What are the possible *causes of inflammatory complications* in our experiments? Aseptic precautions make infection a rarity, but it does sometimes occur. When the pneumonia is intense and accompanied by pleurisy, as occasionally happens, infection is to be presumed, whether or not an organism can be identified. Instances of severe pneumonia were relatively rare. There is no way of telling the exact incidence, since it is only when an animal dies of pneumonia that the diagnosis can be made, but a rough idea may be gained from the fact that only 1.7 per cent. of deaths within 3 weeks of injection were due to this cause.

There seems to be evidence of fortuitous irritants in some of the experiments, though their identity is obscure. Little or no attention has been given to them by other experimentalists in the field of pneumokoniosis. We can but enumerate some of the possibilities.

(i) *Temperature of inoculum.*—It was planned to have the inoculum at body temperature at the time of injection but this condition was not rigidly adhered to and it is probable that, in many instances, the inoculum had cooled to room temperature (55°–65° F.) by the time it was used. Whether or not this chilling would materially augment the chances of inflammation is a matter of conjecture.

(ii) *Reaction of inoculum.*—The pH of the inoculum was usually 7, and there was little possibility of significant variation in either direction. But no rigid control of pH was exercised, and it may be that even very slight variations had an appreciable effect upon the tissue reaction. The work of Menkin and Warner (1937) suggests that the cytology of a focus of inflammation, as well as the fate of the exudate (whether it resolves or organizes), is greatly influenced by the local pH.

(iii) *Body temperature of animals.*—Temperatures were not taken at the time of injection. We tried to ensure that the animals were kept warm, but it is possible that the body temperature was sometimes subnormal, particularly as some animals were anaesthetized with nembutal or urethane intraperitoneally an hour or two before the operation and were allowed to lie in an open cage at room temperature; the animals were also exposed to room temperature while recovering from the anaesthetic after the operation. If the day was cold, chilling might occur in spite of normal precautions.

(iv) *Anaesthetic.*—If nembutal or urethane was used (intraperitoneally), it was often necessary to supplement it with ether inhalation at the time of the operation. In the later experiments, it was found to be more effective and convenient—as well as less hazardous to the animal—to use ether alone. Thus, in most of the experiments, inhalation of ether from a mask was employed right up to the moment of injecting the dust inoculum, and small amounts of ether vapour would inevitably accompany the dust into the lung parenchyma. Possibly this ether vapour was sometimes sufficiently irritant to augment the “irritant-coefficient” of the dust, to the point of provoking an inflammatory reaction.

(v) *Aspiration of mucus.*—We know from histological examination that varying amounts of mucus were carried down with the inoculum into the air sacs, and it may be that this, too, augmented the irritant-coefficient.

(vi) *Obstruction of bronchioles.*—Obstruction of bronchioles by the inoculum not infrequently resulted. Even if relieved in the course of a few hours or days, a temporary obstruction of this sort might favour the inception of inflammatory change in the shut-off lobule or lobules.

(vii) *Pressure of inoculation.*—The pressure under which the inoculum was forced into the lungs might influence the initial reaction. The inoculum was expelled from a syringe into the trachea as quickly as possible, and under some pressure, in order to secure an optimum distribution in the lungs. We do not know what effect, if any, this would entail in regard to tissue reaction, but mention it as yet another factor which possibly complicated the results.

The incidence of initial inflammatory change is roughly proportional to the silica content of the dust, and roughly inversely proportional to the coal content. Exceptions to both these rules are not infrequent, and may be taken as evidence of complicating factors such as those mentioned above.

(3) *Organization of dust deposits.*—At first, the dust is but loosely housed in the innumerable koniophages which appear so promptly, as if from nowhere; but sooner or later most of the particles become incorporated in a fixed fabric

of connective tissue. This process, by virtue of which the dust is overrun and ensnared by connective tissue, is perhaps best described (in terms of general pathology) as a form of organization.\* In an organized dust deposit the koniophage gives place to the dictyocyte,† whether by transformation or replacement is not clear, and the aggregation becomes reinforced by a network of reticulum.

For the purpose of this report, "organization" connotes the process by which dust becomes fixed or effectively retained in the lung. It represents a transformation from a temporary to a more or less permanent tissue change, by virtue of which the dust becomes finally anchored by fixed tissue cells and fibres. It is, perhaps, the most crucial step in the whole reaction, for upon it largely depends the ultimate form and severity of the lesion. It is a process subject to notable variations, not only in histological features, but also in tempo and intensity.

Organization proceeds in one of two ways: either (a) as a simple *foreign-body reaction*, or (b) by organization of inflammatory exudate such as follows non-resolution of pneumonia—*carnification*. There is, perhaps, no very sharp line of demarcation between them; they overlap to some extent, and sometimes elements of both are present in individual lesions. For the most part, however, they are readily distinguishable, for there is one important feature which sets them apart, namely, inflammatory change. In the former, this is minimal or entirely lacking; in the latter it is an essential antecedent.

(a) *Organization by foreign-body reaction* is a simple, slowly progressive change, originating *de novo* in response to the presence of inert particulate matter, without the impetus of inflammation to start it off. It is characterized by the elaboration of dictyocytes in minimal numbers and their attendant reticulin fibrils, likewise minimal in quantity. Development of this reaction is by imperceptible stages. Very fine reticulin fibrils begin to make their appearance at two or three weeks, forming loose snares for the dust-laden phagocytes (Figs. 9 and 13), which eventually become fixed among the fibres. The density of the fibrillar reaction is never very marked in this type of organization; it never approaches the density commonly found in the "carnification" type of lesion, but a gradual increase of fibres may occur in these deposits which have become stationary, up to about the sixth month. After that, the only apparent change is a slight shrinking of individual dust deposits, due partly to decreasing cellularity, partly to further dispersal of dust particles and perhaps also to contraction of the fibres.

A familiar example of the same sort of reaction is to be found in ordinary tattooing of the skin, in which fine particles of inert, insoluble pigment (mostly India ink and carmine) are lodged in the subcuticular tissues. Many of our dust samples, particularly the coaly ones, provided no more reaction than this (Fig. 13).

Commonly, though by no means invariably, *foreign-body giant cells* also appear (see p. 41), engulfing clusters of particles, and persisting unaltered throughout the experiments, ensnared by reticulum fibres (Figs. 12 and 17). They often blend with nodular dust deposits (of which they form a part), often in such a way as to obscure the structure of the giant cell, rendering it difficult to identify except by the practised eye. As in man, the formation of giant cells seems to be an irreversible reaction in the rodent lung; when present, they are part and parcel of the permanent tissue change. Their incidence is consistent in any given experiment; that is to say, if they are present in one animal, they are almost certain to be present in more or less equal numbers in the others.

It is important to remember that giant cells are not a necessary feature of the foreign-body reaction. They are practically never found in tattooing. Dust reticulation (reticulosis, see p. 41) in the human lung is another example of simple foreign-body

\* Definition from medical dictionary: "The act of becoming possessed of an organic structure."

† From *δίκτυον*, a network; a pathological cell, the mother cell of reticulin in dust reticulation (reticulosis, see p. 41), it is fixed or mobile, similar to the fibrocyte or histiocyte; derived from the cells of the reticulo-endothelial system.

reaction unaccompanied, in the great majority of cases, by giant cells (see *M.R.C. Special Report Series No. 243*). Thus, the reaction to certain dusts is often classified as of foreign-body type though there are no giant cells.

The incidence of giant cells has a definite correlation with particle size; it varies with the percentage of particles over  $12\mu$  in any given dust sample. Yet, when present, these cells also contain clusters of small particles.

(b) *Organization by granulation tissue* is preceded by acute inflammatory change. In the absence of inflammation, as indicated above, organization proceeds only as a foreign-body reaction, or not at all. On the basis of our experimental findings, it may be stated as an invariable rule that whenever pneumonia supervenes in a mineral-dusted lung, resolution is greatly restricted; indeed, it rarely occurs; instead, organization, leading to carnification, is seen.

There is an almost complete absence of polynuclear leucocytes, and this may contribute to non-resolution, for it is the polynuclear cell which conveys the proteolytic enzymes without which there can be no liquefaction of a fibrinous exudate and therefore no resolution. Organization begins a good deal earlier than in the foreign-body reaction. Reticulin fibrils begin to appear as early as the third day; and the fibrillar network rapidly increases in density, sometimes reaching a maximum as early as three weeks after injection; more commonly, it goes on gradually increasing till about the third month.

The individual lesion consists of a compact nodule of dust-laden cells filling a group of contiguous air sacs near the mouth (alveolar duct) of the lobular unit. Fibrocytes or dictyocytes criss-cross the nodule, following along shreds of fibrin; some of them apparently arise from the walls of the alveolar duct. Intercellular reticulin fibrils appear at the same time, ultimately forming a tangled network, the density of which varies with different dusts. As the fibrils increase, the cellularity of the nodule decreases. These fibrils are quite invisible in ordinary haematoxylin-eosin preparations; silver impregnation is required to differentiate them. The appearance of a nodule in a haematoxylin and eosin-stained section is quite deceptive (Figs. 3, 25). It is a pale eosinophilic structure like a hyalinized mass, in which cell outlines are lost; the dust particles, if they be colourless like quartz, are also invisible. One is frequently surprised to find a heavy mineral deposit as well as a dense fibrillar network completely hidden away in this simple-looking nodule; they may be brought to light readily enough by micro-incineration and by silver staining, respectively.

(4) *Fibrosis*.—This term, as usually employed, connotes collagen formation, and in this sense is not applicable to experimental pneumokoniosis. But if used in a wider sense so as to include *reticulin fibrosis*, it may also be applied correctly to dust reticulation (reticulinosi, see p. 41) in both man and animals.

The type of fibrosis with which we are concerned is composed almost exclusively of very fine fibrils, namely reticulin fibrils (see p. 33), which are differentiated only by silver impregnation. These fibrils are the precursors of collagen, but do not necessarily develop into collagen; they may remain indefinitely as reticulin, and this is their usual fate in experimental pneumokoniosis.

When organization takes the form of a foreign-body reaction, as in tattooing, the amount of reticulin elaborated is negligible; it is barely enough to secure fixation of the dust (Fig. 13). In the carnification type of organization, seen in these lungs, the dust deposits are much heavier and the fibrillar network may be and often is very dense (Figs. 3, 4, 25, 26, 28, 29); yet even here it is still quantitatively proportional to the amount of dust. There is no disproportionate (redundant) fibrosis like that which characterizes human silicosis, nor evidence of that rather striking encapsulation phenomenon to which Stewart and Faulds (1934) have drawn attention in haematite miners' pneumokoniosis. For that matter, neither is there any real collagen formation.



Reticulin fibrils tend to become coarser with age, and there is an apparent hyalinization in the older lesions which looks quite like collagen in ordinary sections: when, however, the silver stain is applied, a rich network of fine fibrils is still found, completely hidden in what appeared to be a hyalinized deposit of collagenous fibrosis (Figs. 3, 25). For purposes of definition, a fibre is still reticulin as long as it remains argentophilic; it does not become collagen until it loses its affinity for silver. On this basis, the fibrosis encountered in our animals, even in the experiments of longest duration, was exclusively of reticulin type.

As in organization by foreign-body reaction, the nodules of the carnification type tend to become smaller with age, due partly to loss of cellularity and to cell shrinkage, and partly to contraction of fibres. In addition, there occurs another change, namely recanalization, characterized by the appearance of small lacunae in the nodules, apparently representing an attempt to re-establish air sacs and air passages which have been obliterated. Minor degrees of this process are to be seen as early as the sixth month (Fig. 3), but it is only after 18 months or 2 years that it becomes well marked. In one quartz-dusted guinea-pig which survived for 2½ years, the nodules had become so porous as to be scarcely recognizable. They looked and felt like nodules on macroscopic examination, but under the microscope they were at first quite difficult to identify, for the appearance was that of focal areas of fibrous thickening of alveolar walls. The re-opened air spaces were lined by a conspicuous cuboidal epithelium so that the appearance at first suggested multiple small fibro-adenomata. But micro-incineration proved them to be silicotic, and silver staining showed the fibrosis to be still of reticulin type.

Confluent fibrosis was not often encountered, except in those experiments where mixtures of quartz and other dusts were employed (group j, Table XX). At first sight, it seemed to be the result of large areas of lung having been filled up completely with dust, but the explanation is probably not so simple as that. There was always a heavy silica ash in these lesions—hence they are intimately related to the presence of silica—but confluent carnification had obviously occurred, and this points to the probability of an extensive inflammatory reaction having taken place in the early stages. Finally, contraction of fibrous tissue had probably helped to obliterate any remaining air spaces in the affected area, leaving a solid mass of dust-ridden airless tissue (Fig. 30). (There is apparently no attempt at recanalization in the larger lesions where more than one lobular unit is involved; see above.)

Though massive areas of fibrosis were infrequent, minor degrees of confluence were not uncommon. Large composite nodules, formed by the coalescence of several smaller ones, occurred in some of the coal dust experiments (Fig. 21), and here again complicating factors are to be sought, for the usual reaction in a coal-dusted lung is dispersal rather than coalescence of particles. The complicating factor is almost certainly inflammatory change (of variable or indeterminate cause), which gives rise to larger foci of organization than would otherwise occur.

#### *Comparison of the Effects of Coal and Stone Dust*

In all the dusts under experiment, there are two principal components, namely coal and siliceous minerals. Some dusts consist almost entirely of one or the other, but most are a mixture of both in varying proportions. Coals of different rank (from anthracite to bituminous), as well as silica in the form of quartz and silicate minerals, are represented in the series, and these, in addition to the mixed dusts, have been put to the test of experimental pathogenicity. It may serve as a useful introduction to the results as a whole if we first

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indicate briefly in tabular form the usual histological reactions to each of the two main substances:—

*Contrast in Tissue Reaction to Coal and to Silica\**

<i>Histological change.</i>	<i>Coal.</i>	<i>Silica.*</i>
Initial inflammation .. ..	Absent .. ..	Present.
Organization .. ..	Foreign-body type .. ..	Carnification.
Distribution .. ..	More scattered .. ..	Nodular.
	Interstitial .. ..	Intra-alveolar.
Reticulin fibrosis .. ..	Grade 1 .. ..	Grades 1 to 4.

\* This term includes quartz (group a), silicates of the shales (group c), of the coal seam mineral matter (e), of the air-borne dusts (f) and of the micaceous minerals and kaolin of group (h).

Exceptions to some of the above contrasts are not infrequent. Initial inflammation is sometimes present with coal and absent with silica (pp. 44 and 46). As regards distribution, the contrast sometimes breaks down: coal lesions may retain a nodular arrangement for months (Fig. 8) instead of scattering into smaller deposits, while silica occasionally scatters more than usual (Fig. 22). This may be a matter of particle size; very small silica particles may be dispersed more readily than large-sized coal particles. Again, it is often difficult to tell whether a dust deposit is interstitial or intra-alveolar (Fig. 9); originally intra-alveolar, particles may end up in interstitial spaces without having shifted from their original location, due to obliteration of the air spaces in which they lay, and the blending of alveolar septa with intra-alveolar connective tissue.

Exceptions notwithstanding, there is a well-marked contrast, particularly in the type of organization and the density of reticulin fibrosis characteristic of each. With the coals, fibrosis never rises above the minimum (grade 1), whereas with the silicas it ranges between grades 1 and 4.

A significant difference between the two, not mentioned in the table, lies in the different effect each exerts upon the konoiphage. This effect is manifest from the first, and probably determines to a large extent the principal differences indicated above. A phagocyte laden with silica tends to lose amoeboid activity and to become inert. Numbers of these cells cluster closely together in or near the alveolar duct, precisely where the dust originally came to rest, and there they remain. This immobilization hinders dispersal and elimination, and causes the silica lesion to remain predominantly intra-alveolar, whereas the coal dust, for the most part, is carried into the interstitial tissues.

Some silica-laden cells do, in fact, migrate, and considerable numbers reach the root glands. Indeed, the impression gained from examining the glands in the series as a whole is that more silica than coal is thus transported (Fig. 29). Investigation of this seeming paradox yields two possible explanations:—(i) Initial inflammatory change is more frequently associated with silica than with coal, and possibly aids the migration by inducing a flow of lymph towards the hilus; (ii) Silica particles in the root glands are very fine (the majority being under  $1\mu$ ); they are smaller by a good deal than the clumps of coal, which consist of numerous particles packed into dense little aggregates, snowball fashion, as a result of their tendency to flocculate.

### *Comparison of Experimental Lesions with Pneumokoniosis of Miners*

A previous report (Belt and Ferris, 1942) gave a detailed account of the histological changes in the lungs of a series of 42 South Wales coalminers, under the headings indicated in the table below. The same classification applied to experimental lesions is set down opposite for comparison:—

*Classification of Histological Changes in Pneumokoniosis :*

<i>Of Coalminers</i>	<i>In Experimental Animals</i>
Dust reticulation (reticulosis)*	Dust reticulosis
Nodulation	Nodulation
Silicotic	
Reticular (reticulin)†	Reticulin
Mixed	
Confluent fibrosis	Confluent fibrosis
Reticular (reticulin)	Reticulin
Mixed	
Koniophthisis	

It is important to compare the experimental with the human lesions, noting the principal similarities and divergencies, especially the latter, for there are certain fundamental differences to be considered.

The tissue changes are simpler and less complicated in experimental than in human pneumokoniosis. Indeed, there is but one type of lesion which need concern us, viz., dust reticulosis. Nodulation and confluent fibrosis appear only as variations of the reticulin reaction, namely as nodular reticulosis and confluent reticulosis respectively. The "mixed nodules", "silicotic nodules" and what we have defined specifically as "silicotic fibrosis" in the previous report do not occur. Heavy collagenous fibrosis is not to be found; indeed, it is questionable whether true collagenous change was present in any of our animals (pp. 37 and 38). Likewise the features of encapsulation and redundant fibrosis, so common in the miner's lung, are almost completely lacking.

There are four factors which cause experimental pneumokoniosis to differ from the human disease, namely: (1) anatomical peculiarities of the animal lung, (2) dosage of dust, (3) shorter duration of exposure to dust, and (4) absence of tuberculous infection.

(1) The rodent lung has much less interstitial tissue (cf. Miller, 1937), and a less elaborate system of interstitial lymphatics, than the human lung. There is, therefore, a correspondingly lesser capacity for interstitial storage of dust.

(2) A single massive dose of dust, such as we employed experimentally, gives rise to stagnant accumulations of dust in the air sacs; whereas in miners who inhale minute quantities from day to day over a period of years, the air sacs are kept relatively clear. The effect of stagnant dust collections is to produce a sort of carnification, not unlike that resulting from the organization of pneumonia, a feature not often encountered in coalminers' pneumokoniosis.

(3) The great chronicity of coalminers' pneumokoniosis cannot be duplicated in laboratory animals, for the simple reason that they do not live long enough. Collagenous transformation of reticulin fibrosis, in so far as it is a matter of time, lacks the necessary interval in which to make its appearance in experimental pneumokoniosis.

(4) Tuberculous and other infective processes play a considerable part in augmenting the effect of dust in man, whereas in experimental animals these influences are absent.

Thus, at the outset, these fundamental variations have to be reckoned with in the animal as opposed to the human lungs: there is a lesser capacity for interstitial storage of dust; carnification plays a more important role; the

\* Owing to criticism of the use of the term "dust reticulation" for the histological change, the term has been altered in this paper to "dust reticulosis".

† It has been pointed out that the term "reticular fibrosis" is sometimes used to describe a certain loose type of collagenous fibrosis; hence, to distinguish the fibrosis described in this paper, the term "reticulin fibrosis" is substituted.

fibrosis is chiefly reticulin rather than collagenous; infective complications are practically non-existent.

*Dust reticulosis in human and experimental pneumokoniosis.*—This condition, first described as a clinical and pathological entity in *M.R.C. Special Report Series No. 243 (1942)* is by far the most significant pathological change common to both human and experimental pneumokoniosis. It is a permanent dust-ridden state of the lungs, with a simple connective tissue reaction of reticular character sufficient only to secure the dust deposits.

Experimental dust reticulosis as seen in our animals is somewhat different from that of coalminers. It does not necessarily correspond with X-ray reticulation.\* It is more uneven in distribution, as a result of the dust being administered in a single, massive dose; and it is not confined to the interstitial tissues of the lung (see above). Some of the dust gains access to the interstitium via lymphatic channels (this is especially true of coal), but much of it tends to remain in the air sacs, where carnification commonly overtakes it.†

## 6. RESULTS OF INDIVIDUAL EXPERIMENTS

It may be of interest to note that the histological results of these experiments were worked out by one of us (T.H.B.) without foreknowledge either of the composition of the dusts (except the quartz), or of the special object which prompted the inclusion in the series of any given sample. This tended to obviate prejudices arising out of preconceived ideas as to the probable outcome of the various experiments.

The histological reactions are set down briefly in Tables XI to XX, opposite details of the corresponding dusts. Those experiments chosen for photographic illustrations are indicated in the final column of the table.

We have relied to a large extent upon the photo-micrographs to convey the nature and extent of the tissue reactions, and have been at pains to make them as widely representative as possible. Each picture may be regarded as representative of any number of similar fields in their respective experiments.

The dusts are grouped according to origin as follows:—

	Table
(a) Pure quartz (control) .. .. .	XI
(b) Strata of mine A3 .. .. .	XII
(c) Clod (shale) strata of other mines .. .. .	XIII
(d) Low ash coal fraction (clean coal) .. .. .	XIV
(e) High ash coal fraction (extraneous mineral matter) ..	XV
(f) Air-borne mine dusts and screened fractions of coal ..	XVI
(g) Raw (unsterilized) mine dusts .. .. .	XVII
(h) Isolated fractions of minerals in mine dusts .. .. .	XVIII
(i) Human lung residues .. .. .	XIX
(j) Artificial mixtures of pure quartz and mine dusts ..	XX

*Group (a). Pure quartz (Table XI).*—Experiments with this dust were in the nature of controls.

The purest available form of quartz was employed, namely Brazilian pebble, a pure rock crystal, 99.8 per cent.  $\text{SiO}_2$ , ground to a fine powder in which 99 per cent. of the particles

\* The original histological definition of dust reticulation in coalminers (*M.R.C. Special Report Series No. 243, p. 220*) runs in part: "a dust-ridden state of the lungs corresponding to X-ray reticulation". Our animals were not X-rayed, but, had they been, it is unlikely that the pictures would have shown X-ray reticulation as described by Hart and Aslett (*ibid.*, p. 48).

† In ordinary carnification of the lung, staining by silver technique invariably reveals a dense fibrillar network of reticulin in the newly-formed intra-alveolar plugs of connective tissue, thus showing that the process of carnification is concerned with an elaboration of reticular tissue.

TABLE XI  
Experiments with Pure Quartz. Group (a) Control

Description of dust.				Duration of experiments.	Histological changes.						
Source.	Designation.	Particle size. <1 $\mu$ 1-5 $\mu$ .	Coal.		Approximate composition.	Initial inflammation.	Effective retention.	Type of organization.	Distribution.	Reticulin fibrosis.	Photomicrograph.
				Quartz. Mica. Kaolin. Carbonates.							
Brazilian pebble	—	per cent. 80 20	—	per cent. 99.8 — — —	mo. 7	Present	per cent. 80-100	Carnification	Nodular with coalescence	Maximal Grade 4	Figures 2 and 3

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were less than  $2.5\mu$  in size. Injected intratracheally into rats, guinea-pigs and rabbits in doses of from 50 to 250 mg., it produces a standard type of nodular lesion, characterized by a dense snare of reticulin fibrils (Figs. 2 and 3), which begins to appear sometimes as early as 3 days after injection, is fully developed at 3 months, and undergoes a slow loss of cellularity, coarsening of fibres, hyaline change and contraction from the 6th month onwards. The standard quartz reaction, both in man and in animals, has been described in some detail in an earlier paper (Belt, Ferris and King, 1940).

The tissue reaction to pure quartz showed a high degree of uniformity: 23 of the 26 rats employed (approximately 88 per cent.) developed characteristic changes; the fully developed lesion (Figs. 2 and 3) may be regarded as a standard with which to compare the tissue reactions to the various other dusts. There are two aspects, in particular, of the quartz lesion which serve as control factors: firstly, the length of time it requires to reach maturity, and secondly, the density of the fibrillar network.

Negative results with quartz, though relatively infrequent, may throw some additional light on the *modus operandi* of silica. The three "negative" animals survived 41, 87 and 120 days, respectively. One was killed; the cause of death in the others was undetermined, but was probably something other than lung disease. The greater part of the dust in these animals remained in koniophages, which were sometimes closely packed and sometimes loosely arranged in their air sacs. The appearance was like that described by Denny, Robson and Irwin (1939) in their quartz plus aluminium experiments, in which quartz produced a negative result, supposedly because of an inhibiting action on the part of aluminium. In our experiments, there was no apparent reason for the failure of organization. It would be incorrect to say that the results were completely negative in these 3 animals; there was, indeed, a modicum of the usual reticulin fibrosis but it was present only here and there, in small isolated foci. Similarly, the "positive" animals were none of them completely positive; frequently patches of unorganized quartz lay side by side with typical nodules (Figs. 2 and 3), the particles still lodged in loose koniophages having undergone no apparent change since the beginning of the experiment.

Failure of quartz to produce its usual fibrillar reaction, other factors being equal, seems to us accountable only on one of two assumptions: (1) that the positive reaction is partly dependent on some adjuvant like acute inflammation, and a negative result is attributable to failure of this adjuvant, or (2) that a negative result is due to the intrusion of some antidotal agent like aluminium, which inhibits the fibrogenic action of the quartz. We shall return to this topic in the commentary. For the moment, it will suffice to point out that what is true of quartz is almost certainly true of other dusts which contain silica. The principal experiments are therefore subject to the same unknown sporadic influence, which is either the absence of an adjuvant or the presence of an antidote. It is a minority influence and does not seriously jeopardize the experiments, since there is a high degree of uniformity in the positive results in a good majority of the animals used in the control tests. But it is well to keep in mind that this interfering influence is apparently at work to some extent in all the animals, affecting a minority of the quartz dust in sporadic fashion even in the "positive" animals.

• *Group (b). Strata of mine A3* (Table XII).—This mine was chosen for investigation stratum by stratum, because it had one of the highest incidences of pneumokoniosis in the South Wales coalfield. Of the 7 principal strata (Fig. 1), stratum A, sandstone, which contains the highest percentage of quartz (71 per cent.) produced the heaviest fibrosis (grade 4). Stratum B, of which further mention is made in the next paragraph, contains 31 per cent. of quartz and produced only a minimal grade of fibrosis. This may be related to the fact that dust A was attended by well marked inflammatory change

TABLE XII  
Experiments with Strata of A 3 Mine. Group (b)

Description of dust.							Duration of experiments.	Histological changes.						
Source.	Designation.	Particle size <1 $\mu$ 1-5 $\mu$		Coal.	Approximate composition.			Initial inflammation.	Effective retention.	Type of organization.	Distribution.	Reticulin fibrosis.	Photomicrograph.	
Mine A 3*	Stratum A †	78	22	—	71	22	2 5	mo. 12	Present	80 to 100	Carnification	Nodular with coalescence.	Grade 4	Figs. 4
Mine A 3	Stratum B	76	24	—	31	51	6 7	8	None	80 to 100	Foreign body	Nodular	Grade 1	5 and 6
Mine A 3	Stratum C	78	22	—	8	77	9 1	12	None	80 to 100	Foreign body	Nodular and scattered	Grade 1	7
Mine A 3	Stratum D	56	42	88	1	2	8 1	8	None	80 to 100	Foreign body	Nodular and scattered	Grade 1	8 and 9
Mine A 3	Stratum E	59	40	96	‡	‡	2 1	8	None	50 to 70	Foreign body	Nodular and scattered	Grade 1	—
Mine A 3	Stratum F	77	22	26	6	60	7 1	8	?	50 to 70	Foreign body	Nodular	Grade 1	—
Mine A 3	Stratum G	59	39	97	‡	‡	2 ‡	8	None	50 to 70	Foreign body	Nodular and scattered	Grade 1	—

\* "High incidence" anthracite mine.

† See Fig. 1 for diagram of these strata, also M.R.C. Special Report Series 244 for more detailed description of dust samples.

shortly after injection, thus affording an impetus to organization which was lacking in the case of dust B, because the latter failed to provoke an initial inflammatory reaction. Further reference to the role of inflammation will be found in the general commentary. Stratum C, containing the highest percentage of micaceous silicate (77 per cent.), produced grade 1 fibrosis. There was, indeed, a marked spread in the degree of fibrosis produced by the stony strata A, B, C and F, but one effect was common to all, namely relative immobilization of phagocytes, by virtue of which a nodular or "bunched-up" intra-alveolar distribution was preserved; whereas the coaly strata, D, E and G, lacking this effect upon the phagocytes, underwent a good deal of scattering.

*Group (c). Clod (shale) strata from other mines (Table XIII).*—Clod, or shale, is the stratum of rock immediately overlying the coal. It contains a high concentration of mica, with lesser quartz and kaolin, and variable small amounts of carbonates and other minerals. This stratum, analogous with B and C of mine A3, is common to two other mines, A1 and S4; A1 is a high incidence, S4 a low incidence mine. It was chosen for experiment, therefore, to see, if possible, if it had the same pathogenicity in each mine. This proved to be the case. The reaction was much the same for each of 3 samples as for stratum B of mine A3, showing only minimal (grade 1) fibrosis (Table XIII and Figs. 5 and 6). There was, however, a relative immobilization of phagocytes, which showed itself in two ways. Firstly, there was a falling off in the degree of effective retention as compared with the other groups, about half the dust remaining unorganized, being simply engulfed in loose-lying koniophages in the air sacs, in much the same way as in the few instances of negative reaction with pure quartz. Secondly, the organized dust maintained a nodular distribution, indicating little movement of the koniophages after they had ingested the dust. This seems to indicate a potential pathogenicity. But fibrogenic propensities were slight, and the dust, therefore, is probably relatively innocuous.

*Group (d). Low ash coal fraction (clean coal) (Table XIV).*—Five samples were examined, from 5 different mines. The object here was to test different varieties of pure coal. Anthracite mines show a higher incidence of pneumoconiosis than bituminous or steam coal mines. Could the coal itself be accountable for this? The results obtained seem to answer this query in the negative, for the tissue reactions were practically uniform for all 5 samples (Figs. 10 to 13). The initial reaction, though accompanied by a good deal of activity on the part of the koniophages, was usually non-inflammatory, i.e., showing no fibrin nor polymorphonuclear leucocytes. The dust deposits, at first compact and nodular, became thinned out and more widely scattered as time went on, lodging mainly in the interstitial tissues, where they produced nothing more than the simplest type of foreign-body reaction—fibrosis grade 1. There was usually evidence of concomitant emphysema, and presumably some decrease in vital capacity. The picture as a whole is not unlike dust reticulation in coalminers as described in *M.R.C. Special Report Series No. 243 (1942)*.

*Group (e). High ash coal fraction (extraneous mineral matter) (Table XV).*—Four samples were examined from the same mines as in group (d) with one exception. This fraction is a by-product of the preparation of clean coal. It is that part of whole coal which, in powdered form, sinks in a fluid of specific gravity 1.60, whereas the part that floats at 1.40 is clean coal. In *M.R.C. Special Report Series No. 244 (1943)*, the mineral part of this material is referred to as the "extraneous mineral matter" of the coal. It forms part of the coal seam, and should not be confused with the mineral matter derived from



TABLE XIII  
 Experiments with Clod (Shale) Strata of Other Mines. Group (c)

Description of dust.						Histological changes.							
Source.	Designation.	Particle size. <1 $\mu$ 1-5 $\mu$	Approximate composition.				Duration of experiments.	Initial inflammation.	Effective retention.	Type of organization.	Distribution.	Reticulin fibrosis.	Photomicrograph.
			Coal.	Quartz.	Mica.	Kaolin.							
A 1*	94LPJ ..	per cent. 82 18	—	per cent. 25 66 7 —	—	8	None	per cent. 40 to 50	Foreign body.	Nodular and scattered.	Grade 1	(See Figs. 5 & 6 for identical lesions)	
A 1	94LP2F..	79 21	—	25 66 7 —	—	8	None	40 to 50	Foreign body.	Nodular and scattered.	Grade 1		
S 4†	83/2'9* C+H+3	76 24	—	16 66 8 9 (Fe)	—	8	None	40 to 50	Foreign body.	Nodular and scattered.	Grade 1		

\* A1 = "high incidence" anthracite mine.

† S4 = "low incidence" steam coal mine.

TABLE XIV  
*Experiments with Low Ash Coal Fractions (Clean Coals). Group (d)*

Sources.	Description of dust.					Duration of experiments.	Histological changes.								
	Designation.	Particle size.		Approximate composition.				Initial inflammation.	Effective retention.	Type of organization.	Distribution.	Reticulin fibrosis.	Photomicrograph.		
		<1 $\mu$	1-5 $\mu$	Coal.	Quartz.	Mica.	Kaolin.	Carbonates.		per cent.					
A 3*	—	per cent. 50 44		99	per cent. — — ½ ½				8	None	80 to 100	Foreign body.	Scattered	Grade 1	Figs. 10
A 1*	94LP ..	35	63	99	— — ½ ½				8	None	80 to 100	Foreign body.	Scattered	Grade 1	—
A 2†	95LP ..	52	45	99	— — ½ ½				8	None	80 to 100	Foreign body.	Scattered	Grade 1	—
S 4‡	—	39	59	99	— — ½ ½				8	None	80 to 100	Foreign body.	Scattered	Grade 1	11
B 2§	—	46	52	99	— — ½ ½				17	None	80 to 100	Foreign body.	Scattered	Grade 1	12 and 13

\* A3 and A1 = "high incidence" anthracite mines.

† S4 = "low incidence" steam coal mine.

‡ A2 = "low incidence" anthracite mine.

§ B2 = "low incidence" bituminous mine.

TABLE XV

Experiments with High Ash Coal Fractions (Extraneous Mineral Matter)\*. (Group e)

		Description of dust.					Duration of experiments.	Histological changes.							
Source.	Designation.	Particle size.		Approximate composition.				Initial inflammation.	Effective retention.	Type of organization.	Distribution.	Reticulin fibrosis.	Photomicrograph.		
		<1 $\mu$	1-5 $\mu$	Coal.	Quartz.	Mica.	Kaolin.	Carbonates.	Other constituents.						
A 3*	—	per cent.			per cent.			mo.		per cent.					Figs.
		55	41	53	3	5	7	8	—	80 to 100	Foreign body.	Scattered	Grade 1	14	
A 1†	94LP	38	62	64	3	12	9	9	—	80 to 100	Foreign body.	Scattered	Grade 1	—	
A 2‡	95LP	46	52	66	1	6	14	8	—	80 to 100	Foreign body.	Scattered	Grade 1	—	
S 4§	—	39	58	37	3	4	5	46	(Fe)	80 to 100	Foreign body.	Scattered	Grade 1	15	

\* Extraneous mineral matter= residue from powdered coal sinking in sp. gr. 1.60.

† A3 and A1 = "high incidence" anthracite mines. ‡ A2 = "low incidence" anthracite mine.

§ S4 = "low incidence" steam coal mine.

the clod and other stony strata adjacent to the coal. It contains much kaolin, whereas the chief siliceous component of clod is mica. The object of testing these samples was much the same as for group (d). The results are also much the same (Figs. 14 and 15). Though these dusts contain up to 84 per cent. of siliceous mineral matter, they produced the same type of reaction as pure coal. There may be a slightly denser fibrillar reaction than with clean coal, but not enough to warrant calling it grade 2 fibrosis. The important thing is that the reaction conforms with the same simple foreign-body type, and the distribution also is similar.

*Group (f). Air-borne mine dusts and screened fractions of coal* (Table XVI).—Fourteen samples were examined, from 6 different mines. This large group consists of selected specimens collected in the various working places of the mines—at the face, in headings and in stalls—and are to be regarded as representative of the dusts actually breathed by the men who contract pneumokoniosis. They were collected in fairly substantial amounts, over many hours, by cloth and labyrinth filters. A full description of their composition and properties will be found in *Special Report No. 244*. Some other samples were collected from the tramways, where the dust had settled. Two high incidence anthracite mines, A1 and A3, were sampled, one low incidence anthracite mine A2, one steam coal mine S4 and two bituminous mines B2 and B3, all low incidence. Mine A3 was more thoroughly investigated than the others in respect of air-borne dusts, as well as of its strata; there were 6 air-borne samples from the mine, corresponding with the dust hazards in various working activities from drilling in rock to loading coal.

The object was to make the samples as representative as possible of the dust the miners inhale, but it must be recognized that they may fall short of this objective. They represent the dust of a few days at most, and it can only be assumed that this is substantially the same as the dust breathed by the men over a period of many years.

It will be seen (Table XVI) that the animal findings cut across the incidence of human pneumokoniosis in the mines concerned, and show some correlation with the siliceous content of the dust. The place of collection seems to affect the result only in so far as it leads to a higher or lower silica concentration in the sample.

Each sample contains a major proportion of coal, ranging from 62 to 94 per cent., and a minor proportion of siliceous minerals, ranging from 5 to 28 per cent. In some samples, mica is the chief mineral, showing that these dusts have been derived in part from the clod; in others, kaolin predominates, as in the extraneous mineral matter from coal seams, group (e).

Nine of these samples produced a simple foreign-body reaction with fibrosis grade 1, similar to that of groups (d) and (e)—Figs. 16 and 17. The average coal content of these 9 samples was 85 per cent., the average silica content 12.5 per cent.

The remaining 5 samples produced something more than a foreign-body reaction. Organization was partly of the carnification type (Figs. 18 to 21), and fibrosis was of grades 2 and 3. These dusts averaged 74 per cent. of coal and 22 per cent. of siliceous minerals.

Inflammatory changes in the early stages (3rd to 6th days) were inconspicuous and difficult to define. It is not possible to state with assurance whether they were more marked in connexion with one experiment than with another.

The results of this group of experiments provide some evidence of a positive correlation between silica content and density of fibrosis. Two of the positive samples have 8 and 4 per cent. of quartz, but the other 3 have only 1 and 2

TABLE XVI  
Experiments with Air-borne Mine Dusts Group (f)

		Description of dust.							Histological changes.						
Source.	Designation.	Particle size.		Approximate composition.					Duration of experiments.	Initial inflammation.	Effective retention.	Type of organization.	Distribution.	Reticulin fibrosis.	Photomicrograph.
		<1 $\mu$	1-5 $\mu$ .	Coal.	Quartz.	Mica.	Kaolin.	Carbonates.							
A 3*	D    ..	per cent.		84	3	per cent.			mo.	None	per cent.	Foreign body.	Scattered	Grade 1	Figs.
		63	33			4	8	1	12		80 to 100				—
A 3*	E    ..	55	42	61	8	10	20	1	12	Present	80 to 100	Carnification.	Nodular and scattered.	Grade 2	18 and 19
A 3*	I    ..	51	46	82	3	5	9	1	12	None	80 to 100	Foreign body.	Nodular	Grade 1	16 and 17
A 3*	2H1 <sub>4</sub>    ..	54	44	82	2	6	6	3	16	None	80 to 100	Foreign body.	Scattered	Grade 1	—
A 3*	2H <sub>3</sub>    ..	55	43	85	1	3	9	1	8	None	80 to 100	Foreign body.	Scattered	Grade 1	—
A 1*	2M <sub>1</sub>    ..	60	38	83	3	5	4	3	17	None	80 to 100	Foreign body.	Scattered	Grade 1	—
A 2†	1S <sub>2</sub>    ..	63	36	92	‡	2	4	1 Sulphates	8	None	80 to 100	Foreign body.	Scattered	Grade 1	—
S 4‡	1U <sub>1</sub>    ..	63	36	87	3	5	3	1	8	None	80 to 100	Foreign body.	Scattered	Grade 1	—
B 2§	1C <sub>1</sub>    ..	55	43	79	3	10	6	1	16	None	80 to 100	Foreign body.	Scattered	Grade 1	—

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TABLE XVI—contd.

		Description of dust.							Histological changes.							
Source.	Designation.	Particle size.		Approximate composition.					Duration of experiments.	Initial inflammation.	Effective retention.	Type of organization.	Distribution.	Reticulin fibrosis.	Photomicrograph.	
		<1 $\mu$	1-5 $\mu$ .	Coal.	Quartz.	Mica.	Kaolin.	Carbonates.								Other constituents.
B 3 §	1B <sub>1</sub>    ..	per cent. 49	49	62	4	16	8	—	Cal- cite.	mo. 16	Present	per cent. 80 to 100	Carnification and foreign body.	Nodular and scattered.	Grade 3	Figs. 20
A 3 *	91US28 **	54	44	85	1	3	7	3	—	8	Present	80 to 100	Carnification and foreign body.	Nodular and scattered.	Grade 2	—
A 1 *	94US10 **	60	38	82	2	5	5	5	—	8	Present	80 to 100	Carnification and foreign body.	Confluent nodules.	Grade 2	—
A 2 †	95US10 **	57	42	84	1	5	6	3	—	8	Present	80 to 100	Carnification and foreign body.	Confluent nodules.	Grade 2	21
S 4 ‡	83US10 **	58	41	94	‡	2	3	‡	—	8	None	80 to 100	Foreign body.	Nodular and scattered.	Grade 1	—

\* A1 and A3 = "high incidence" anthracite mines.

† A2 = "low incidence" anthracite mine.

‡ S4 = "low incidence" steam coal mine.

§ B2 and B3 = "low incidence" bituminous mines.

|| Air-borne mine dusts collected in a labyrinth or cloth filter.

\*\* Air-borne dust settling on tramways; screened through 300 mesh sieve.

per cent., respectively. The silicate content may, therefore, be held accountable, and the experiment be taken as evidence that micaceous mineral matter (chiefly sericite) and kaolin, either or both, may have fibrogenic properties. But some of the negative dusts are higher both in quartz and in mica and kaolin than 3 of the positive ones; which, moreover, do not differ appreciably in composition from the high ash coal fractions of group (e), which produced only minimal fibrosis. There is, then, a certain amount of overlapping in composition between "positive" and "negative" dusts, which will be discussed in the commentary.

*Group (g). Raw (unsterilized) mine dusts* (Table XVII).—Four samples from mine A6 were examined. All the other dusts were autoclaved before injection, in order to destroy bacteria. But the miners themselves inhale unsterilized dust, probably contaminated with living organisms, which may contribute to the development of pneumokoniosis. It was thought advisable, therefore, to experiment with "raw" dusts such as the miners inhale. For this purpose, sweepings\* were taken from various places, both below and above ground, where men and ponies worked. These specimens were thought likely to contain organisms from the sputum of men and droppings of beasts, as well as soil bacteria. Cultures made prior to injection were quickly overgrown with various large bacilli, thought to be soil bacteria. Tests for *Myc. tuberculosis* (T.B.), including guinea-pig inoculation, were negative. Chemical assay was not undertaken, but it was obvious that these dusts were mostly coal; micro-incineration yielded a siliceous residue roughly estimated at from 10 to 15 per cent. Histological reactions were surprisingly mild; there was scarcely any initial inflammatory change, and the end results after 12 months were much the same as for sterilized clean coal; fibrosis was perhaps a little denser, but still within the category of grade 1.

TABLE XVII

*Experiments with Raw (Unsterilized) Mine Dust. Group (g)*

Description of dust.			Duration of experiments.	Histological changes.					
Source.	Designation.	Particle size. <math>1\mu-5\mu</math>		Initial inflammation.	Effective retention.	Type of organization.	Distribution.	Reticulin fibrosis.	Photomicrograph.
A 6*	2R ...	per cent. 60 39	mo. 12	None	per cent. 80 to 100	Foreign body.	Scattered	Grade I	—

\* A6 = "high incidence" anthracite mine.

*Group (h). Isolated fractions of minerals in mine dusts* (Table XVIII).—Five samples, including "fine fractions" from 3 different mines were examined. "Fine fractions" represent a mineral of the mica group which is the main constituent of the shales overlying the coal in several mines. Several samples of shale have already been reported upon in the experiments of groups (b) and (c) (Tables XII and XIII), and it has been shown that they are lacking in fibrogenic properties. "Fine fractions" constitute the bulk of shale, and it is of interest to study them separately, freed from the quartz with which they are combined in the natural state. "Fine fractions" are composed of the

\* Grateful acknowledgment is made to Dr. Norman Spoor for collecting these specimens.

TABLE XVIII  
*Experiments with Isolated Fractions of Mine Dusts. Group (h)*

Description of dust.							Histological changes.							
Source.	Designation.	Particle size. <1 $\mu$ 1-5 $\mu$	Approximate composition.					Duration of experiments.	Initial inflammation.	Effective retention.	Type of organization.	Distribution.	Reticulin fibrosis.	Photomicrograph.
			Coal.	Quartz.	Mica.	Kaolin.	Carbonates.							
A 3*	"Fine† fraction."	per cent. 100 —	—	1	89	10	—	12	None	per cent. 80 to 100	Foreign body.	Nodular and scattered.	Grade 1	—
A 1*	"Fine† fraction."	100 —	—	1	89	10	—	14	None	80 to 100	Foreign body.	Nodular and scattered.	Grade 1	22
S 4†	"Fine† fraction."	100 —	—	1	89	10	—	17	None	80 to 100	Foreign body.	Nodular and scattered.	Grade 1	23
—	Ogofau-hydro-muscovite.§	41 54	—	—	"pure mica"	—	—	13	Present	80 to 100	Carnification.	Nodular	Grade 4	25
—	Kaolin	78 22	—	8	12	77	—	8	Present	80 to 100	Carnification.	Nodular	Grade 2 or 3?	24
					(hydrated silica).									

\* A1 and A3 = "high incidence" anthracite mines.

† Water sedimented shale, nearly pure hydromica.

|| A "commercial" kaolin.

† S4 = "low incidence" steam coal mine.

§ A hydromica from Ogofau, Carmarthenshire.



micaceous fraction of shale which is often referred to as South Wales sericite and came in for a good deal of attention through the work of Jones (1933). Dr. Nagelschmidt prepared the samples used in these experiments. They contain little kaolin and are almost free of quartz. The histological reaction to these "fine fraction" specimens was uniformly bland in all 36 animals involved, ranking as the slightest in the whole series (Figs. 22 and 23). Phagocytosis took place briskly enough, but there was no initial inflammatory change and the koniophages were not immobilized to the same extent as with other siliceous dusts. Many heavily laden cells reached the root glands, but on the whole their movement was somewhat restricted, and they tended to cluster closely together in small groups (Fig. 22) within the air sacs, with the result that less scattering occurred than in the case of coal dusts. The most striking feature was the apparent indifference of the fixed tissues to the dust; practically no fibres reached out to secure the deposits. Even in the root glands after 12 months there was no appreciable elaboration of fibres in or about the heaviest concentrations of particles (Fig. 23). This indifferent reaction was in marked contrast with that now to be reported.

The next sample, a sericite from an extraneous source,\* was included as a control. It produced a reaction indistinguishable from that of quartz (Fig. 25). This result is at variance with the previous work of several authorities who report little pathogenic effect from sericite (Fallon and Banting, 1935; Lemon and Higgins, 1935; Miller and Sayers, 1936; Cummins, 1937; Gardner, 1938b), and calls for a thorough checking of the composition of the sample, before our results can be put forward as evidence of the pathogenicity of sericite. Since exact data as to the complete composition of this sample are not available, we cannot accurately evaluate our finding. It may be that the sample contained some free silica. Or the explanation may be found in the previous treatment of the dust with acid, a subject which will be dealt with in the succeeding report (cf. p. 87).

The sixth sample was kaolin. This dust, derived from an extraneous industrial source,† was employed for want of a suitable specimen of pure kaolin from South Wales mines. It was not possible to isolate kaolin even in moderately pure form from South Wales material, though it was present in appreciable amounts in practically all specimens except those of pure coal. Because of its being one of the principal constituents (along with coal, quartz and mica) of the many samples, it was thought desirable to make it the subject of a separate experiment. The sample obtained, though extraneous, was regarded as sufficiently similar to South Wales kaolin to warrant its inclusion in the series. The histological result (Fig. 24) showed a nodular distribution, organization of the carnification type, but with some giant cells taking part to cope with the larger fragments, and fibrosis estimated to be of grade 2 or possibly grade 3 density. The phagocytes were definitely immobilized by this dust, and altogether the evidence points to its having a fairly high degree of pathogenicity; this, however, may be due to the 8 per cent. of free silica contained in the sample. An experiment of this kind with but a single sample of kaolin and that obviously not pure, is of course inconclusive. The weight of evidence from other sources points to kaolin being non-pathogenic (Kettle, 1934b; Middleton, 1936; Gardner, 1938b) and that evidence cannot be seriously challenged from the results of this experiment.

*Group (i). Human lung residues (Table XIX).*—The two samples under this heading were prepared by the method of Nagelschmidt and King (1941). This consisted in destruction of the tissue by warm hydrogen peroxide, low temperature combustion of the coal, and extraction of non-siliceous inorganic matter with cold dilute acid. One of the specimens was from a man who had silicotic nodulation from working in sandstone (hardheader). The other was from a collier who had been exposed during his working life to the mixtures

\* A powdered sample of hydromuscovite from Ogofau, Carmarthenshire, kindly supplied by Dr. A. Brammall and described by Brammall, Leech and Bannister (1937).

† The kaolin was a specimen of industrial origin, kindly given us by Dr. Brammall. In addition to kaolin, it contained about 8 per cent. of hydrated free silica and 12 per cent. of mica.

TABLE XIX  
 Experiments with Human Lung Residues.\* Group (i)

Description of dust.		Approximate composition.						Duration of experiments.	Histological changes.					
Source.	Designation.	Particle size. <1 $\mu$ 1-5 $\mu$	Coal.	Quartz.	Mica.	Kaolin.	Carbonates.		Initial inflammation.	Effective retention.	Type of organization.	Distribution.	Reticulin fibrosis.	Photomicrograph.
A 3	P.170. Hard-header.	per cent. 80 20	—	per cent. 20 37 40	—	—	—	mo. 12	Present	per cent. 80 to 100	Carnification.	Nodular	Grade 4	Figs. 26
A 3	P.184. Collier	80 20	—	15 32 50	—	—	—	12	?	80 to 100	Foreign body.	Nodular and scattered.	Grade 1	27

\* Dried, powdered lung digested with H<sub>2</sub>O<sub>2</sub>, heated at 380° to destroy coal and extracted with dilute acid to remove non-siliceous matter..

of anthracite coal and shale which are represented in the air-borne dusts of mine A3 (group *(b)*); this man had dust reticulation (reticulosis, see p. 42) and confluent fibrosis.

The sample from the "hardheader" (Fig. 26) produced a well marked finely nodular fibrosis, distinctly palpable in the gross, which proved to be of a density approaching that induced by quartz, viz., grade 4 (Fig. 26). The initial reaction following injection was of a mild, but definite acute inflammatory nature. The result of this experiment is contrary to that of somewhat similar experiments performed by Simson (1937), who found that silica recovered from a human silicotic lung was non-fibrogenic in animals. In a later communication, however, Simson and Strachan (1940) showed that human silicotic lung residue was fibrogenic in animals after it had been well digested with sulphuric acid, which possibly removed some inactive coating from the particles.

The second experiment yielded a result which, in striking contrast to the first, was characterized by minimal fibrosis (Fig. 27). The dust did, however, immobilize the phagocytes, which implies that it is not without a potentially pathogenic effect. Here again is the suggestion that absence of an initial inflammatory change determines failure of fibrogenesis, for no appreciable inflammation was present in three animals examined on the fourth day after injection.

*Group (j). Artificial mixtures of pure quartz and mine dusts (16 samples) (Table XX).*—The object of these experiments was to attempt to imitate, with simple and complex mixtures of known composition, the mixed dusts breathed by the workers in the various parts of the mines, and to test the effect of one type of dust on the reaction produced by another.

This group of experiments provides, in effect, a limited test of the Haldane theory of the action of "adulterant dusts" in modifying and inhibiting the usual effect of quartz, and the results lend some further evidence to the theory. Results of experiments with low ash (clean) coal (group *(d)*, p. 46) showed no appreciable difference in the reactions to anthracite, bituminous and steam coal. Similarly, in group *(e)*, the high ash coal fractions from each of these types of mines showed a more or less uniform tissue reaction, as did the clod (shale) strata (group *(c)*) from the different mines. There is, therefore, no indication in the results so far of the reason for a higher incidence of pneumoconiosis in anthracite than in bituminous or steam coalmines. One question, however, raised by Professor Douglas (1942) has not as yet been touched upon. Does bituminous coal activate or stimulate the koniophages in the presence of quartz in such a way as to prevent effective retention, while anthracite fails in this respect? By administering mixtures of pure quartz with clean coal of each type it was hoped to put this latter question to the test in the final group of experiments now to be recorded. The following is a brief account of the histological findings:—

#### *Quartz and coal dusts (Table XX)*

1. *Initial reaction.*—Half the animals examined 3 to 6 days after injection showed mild but definite acute inflammatory change. Some others were border-line in this respect, with slight but equivocal evidence of inflammation, and the remainder (approximately 25 per cent.) were apparently negative, presenting merely uncomplicated phagocytosis. The incidence of acute inflammatory reaction following injection appears to bear no significant relation to the type of coal mixed with the quartz.

2. *Effective retention.*—In seven experiments a considerable proportion of the quartz, averaging approximately 50 per cent., remained unorganized, lodged in loose-lying koniophages in the air sacs as long as 18 months after injection (Fig. 28), indicating definite inhibition of fibrogenesis. This effect

TABLE XX  
Experiments with Artificial Mixtures of Pure Quartz. Group (j)

Description of dust.		Approximate composition.					Duration of experiments.	Histological changes.					
Source.	Designation.	Coal.	Quartz.	Mica.	Kaolin.	Carbonates.		Initial inflammation.	Effective retention.	Type of organization.	Distribution.	Reticulin fibrosis.	Photomicrograph.
		per cent.					mo.		per cent.				Figs.
A 3*	Quartz and clean coal	83	17	—	—	—	18	Present	40 to 60	Carnification and foreign body.	Confluent and scattered.	Grade 4	28
A 1*	Qz+94 P ..	83	17	—	—	—	17	Present	60 to 80	Carnification and foreign body.	Confluent	Grade 4	—
A 2†	Qz+95LP ..	83	17	—	—	—	17	Present	80 to 100	Carnification and foreign body.	Confluent and scattered.	Grade 4	29
S 4‡	—	83	17	—	—	—	15	Present	80 to 100	Carnification and foreign body.	Nodular and scattered.	Grade 4	—
B 2§	—	83	17	—	—	—	15	?	60 to 80	Carnification and foreign body.	Confluent and scattered.	Grade 4	—
B 3§	—	83	17	—	—	—	18	None	40 to 60	Carnification and foreign body.	Confluent and scattered.	Grade 4	—

A 3*	Quartz and air-borne dusts Qz+I ..	68	19	4	8	1	13	None	80 to 100	Carnification and foreign body.	Nodular and confluent.	Grade 4	—	
A 3*	Qz+2H <sub>2</sub> ..	63	26	3	7	1	12	?	80 to 100	Carnification and foreign body.	Confluent and scattered.	Grade 4	—	
A 1*	Qz+2MI ..	62	27	4	3	3	12	Present	80 to 100	Carnification and foreign body.	Nodular and confluent.	Grade 4	—	
A 6*	Qz+2R ..	41	51	4	3	‡	12	Present	80 to 100	Carnification and foreign body.	Nodular and confluent.	Grade 4	—	
B 2‡	Qz+1C1 ..	59	27	8	5	1	12	?	60 to 80	Carnification and foreign body.	Confluent and scattered.	Grade 4	—	
B 3‡	Qz+1B1 ..	46	28	12	6	8	12	Present	80 to 100	Carnification and foreign body.	Nodular and confluent.	Grade 4	30	
<hr/>														
	Quartz and clod (shale)													
A 3*	Qz+Clod ..	—	24	64	8	1	8	None	20 to 40	Foreign body.	Scattered	Grade 1	—	
A 3*	QZ+Clod ..	—	31	58	7	1	16	None	20 to 40	Foreign body.	Diffuse	Grade 1	31	

\* A1, A3 and A6 = "high incidence" anthracite mines.  
‡ S4 = "low incidence" steam coal mine.

† A2 = "low incidence" anthracite mine.  
§ B2 and B3 = "low incidence" bituminous mines.

was in no case uniform throughout a given lung. It was more marked in connexion with clean coal than with the air-borne dusts, but amongst the clean coals there was no apparent difference between anthracite and bituminous (compare columns 1 and 6 of Table XX).

3. *Organization* was chiefly of the carnification type but invariably mixed with foreign-body reaction: large numbers of giant cells participated, concerning themselves almost exclusively with coal particles. In column 7 of Table XX it will be seen that all samples, whether the coal was of one type or another, produced approximately the same type of organization, characterized by a mixture of carnification and foreign-body reaction.

4. *Distribution*.—Three terms are used (in column 8 of Table XX, as in other Tables and in the text) to designate the commonest types of distribution, viz. 1. Nodular, as illustrated in Figs. 2, 4 and 5; 2. Scattered, as in Figs. 10 and 12; and 3. Confluent, as in Fig. 30. All three types are commonly present together, but only the most prominent are indicated in the table, and where two types of distribution are listed, the predominant type is, of course, given first. The usual nodular distribution of quartz was very noticeably altered in favour of a more diffuse reaction. The commonest lesion was a diffuse thickening of the alveolar septa accompanied by partial or complete filling of air sacs with organized clumps of dust-laden cells, the whole resembling an organized pneumonia. This change comes under the term "confluent" in column 8 of Table XX. Next in frequency were massive confluent lesions (Fig. 30), a more solid induration than that just described, in which the alveolar architecture was completely superseded. A modified type of nodule was not uncommon; such nodules were small and ill-defined, tending to coalesce; they contained coal as well as quartz and often had giant cells incorporated in them; they remained more cellular than the nodules produced by pure quartz, seldom undergoing hyaline change (Fig. 28). Quartz and coal were for the most part fairly intimately mixed, though sometimes the coal became segregated and not infrequently quartz alone reached the root glands, possibly because of its smaller particle size. Wherever segregation occurred, the quartz, left to itself, usually produced its customary effect.

A glance at Table XX, column 8, will show the high incidence of confluent lesions in these experiments, the like of which is not to be found in any of the other groups (see below). But again, no co-relation appears to exist between the type of coal, whether anthracite, bituminous or steam, and the character or extent of the lesion.

5. *Fibrosis*.—Wherever the quartz had undergone organization, whether in the presence of coal or not and whatever the type of coal, fibrosis was heavy and invariably of grade 4, though perhaps not quite so intense, on the whole, as in the pure quartz experiments (Figs. 28, 29 and 30).

No variations in the dust lesions were found to correspond with variations in the type of coal used in the mixed dusts. It should be stated, however, that such negative evidence as has been adduced regarding this matter is inconclusive, for the number, range and duration of these few experiments are perhaps too limited to lead to a decisive opinion on the relative pathogenic effects of anthracite and bituminous coal.

Pursuing this question in another way, experiments were undertaken with tissue cultures. Pure samples of anthracite and bituminous coal prepared by Mr. Hicks were dusted over a series of tissue cultures (unpublished work by Dr. Imrich Friedmann and Dr. Belt). The results failed to show any clear difference in the reaction of living cells observed from day to day over a period of 28 days to particles of anthracite as compared with bituminous coal, although phagocytosis was in fact slightly more active with steam coal particles.

Altogether, the reactions induced by these mixtures afforded an approximation to the confluent consolidation of coalminers' pneumokoniosis, which is closer to the human disease than any other analogy in the whole series of experiments. Here is perhaps some indication of the way in which the confluent

consolidation of coalminers' pneumokoniosis develops, namely as a modification of the usual silica reaction induced by the intermingling of coal, which has the effect of spreading the reaction more diffusely and more unevenly than is usual in typical silicosis. But other factors are probably concerned also; one cannot escape the impression that confluent areas represent parts which were swept by inflammatory change in the early stages of the reaction.

Our experiments with artificial mixtures of coal and pure quartz are perhaps open to the criticism that a uniform and intimate mixture of the two dusts was not effected. Similarly, the recently published results of experiments with quartz and aluminium (Belt and King, 1943) are open to the same criticism. It is difficult to obtain an intimate and even mixture of coal and quartz, or of aluminium and quartz, because neither coal nor aluminium is readily suspended in a fluid medium; they tend to flocculate and to resist dispersal. In addition, they are much lighter than quartz, hence the latter settles out more rapidly. But shale mixes well with quartz, as it is of similar specific gravity. This may account, in part, for the more marked effect of shale than of aluminium in forestalling the effect of quartz in our experiments.

One experiment in this series merits special note—namely, that in which raw unsterilized coal was mixed with quartz. Considerable numbers of living soil bacteria were present in this sample (see group *g*). The quartz reaction here reverted to a more typical form, with dense hyalinized nodules tending to coalesce. There were 48 animals in this experiment (really four experiments, as four different coal samples, the same as in group *g*), were employed) and the majority showed it. The suggestion is that bacteria perhaps contributed to a heavier quartz effect. The experiment is, of course, not conclusive. But if the bacteria did, in fact, contribute it might be by effecting some dissolution of the quartz. Possibly living organisms in the lung may facilitate dissolution of silica.

Had time and circumstances permitted, it would have been well to work out the bacterial flora of the dust samples, and to test the effect of these organisms on silica solubility *in vitro*. The possibility of bacteria accelerating silica solubility is important in relation to human silicosis. For example, there is much to suggest that *Myc. tuberculosis* (T.B.) acts in this way. Experiments aimed at testing this hypothesis *in vitro* are attended by serious difficulties, e.g. no bacteriological media are silica-free. Experiments by the authors and Dr. J. D. MacLennan gave some evidence that quartz dissolved more readily in the medium of rapidly growing cultures, than it did in controls where it was suspended alone in the sterile medium. This effect was not always observed, however, and the result was considered to be equivocal.

#### *Quartz and clod (shale) (Table XX)*

The reaction to mixtures of quartz and shale is of special interest, because shale dust has long been regarded as a protective agent in the mines—protective not only against explosions but also, according to Haldane, against silicosis. Our experiments with the shale alone (group *c*) showed it to be of low fibrogenic propensities. We then tested its ability to modify the effect of quartz.

The results were quite striking. A large proportion, probably as much as 70 per cent., of the dust remained unorganized up to the end of the experiment, 16 months after injection, resting simply in loose-lying koniophages in the air sacs (Fig. 31). Large areas of consolidation were thus produced, and in some parts the cellular collections were lightly reinforced by reticulin fibrils, but the usual quartz reaction was completely lacking even in the root glands, to which, by the way, only a small amount of dust had been transported. Many of the dust-laden koniophages showed a pale foamy cytoplasm, due to fatty change, and cholesterol crystals were frequently observed in the denser collections, apparently derived from degenerating koniophages.

## 7. DISCUSSION

One of the principal objects of our investigation was to determine, if possible, what properties make a mineral dust fibrogenic. In surveying the results from this point of view, it is apparent that fibrogenesis over and above the level of a simple foreign-body reaction is apparently attributable to silica. The results also suggest that certain silicates as well as quartz may possess this property, although the findings in regard to the silicates are somewhat

contradictory. One group of silicates which we shall put under the designation of shale, so far from being fibrogenic, apparently inhibits fibrogenesis in the more pathogenic varieties of silica.

All gradations of fibrogenic activity are represented amongst the large number of siliceous dusts under experiment. It will be useful to divide them into two groups called, for convenience, positive and negative, including in the former those producing grades 2, 3 and 4 fibrosis and in the latter, those leading only to grade 1 fibrosis.

<i>Positive fibrogenic siliceous dusts</i>	<i>Negative fibrogenic siliceous dusts</i>
[Quartz]*	
Stratum A, mine A3	Strata B, C, D, E, F and G of mine A <sub>3</sub> , shale from other mines
Certain air-borne dusts	Other air-borne dusts
[? Kaolin, "purified, washed with acid"]	Extraneous mineral matter from coal
[Ogofau hydromuscovite]	Fine fractions mica
Human residue A (hardheader)	Human residue B (collier)
Mixture of quartz and coal dusts	Mixture of quartz and clod

A significant distinction between the positive and negative groups of dusts is the incidence of free silica in the former and of shale in the latter.

Shale is a combination of silicates with other minerals. Its chief constituents are quartz, mica and kaolin. Carbonates may be present in varying amount. Minor constituents are chlorite, biotite, rutile, calcite and ankerite. The nature of the factor in shale which is antidotal to quartz is not known. It has not been possible to test the several constituents separately in a pure form, but the two principal components, mica and kaolin, are known to depress silica solubility (King, McGeorge and Roman, 1938), and have been studied biologically by several workers. Brammall and Leech have considered a possible antidotal action of the free iron oxide and alumina contained in these shales.

Attempts to prepare mica and kaolin, free from contaminants, from the shales of South Wales have not been completely successful. In the "fine fractions", the mica has been concentrated until it constitutes 89 per cent. of the whole. This material is of very low solubility, depresses the solubility of quartz (King, pp. 69-94 below), and produces only minimal fibrosis. It is possible that the more soluble (and therefore, presumably, the more pathogenic) components of the mineral mixture were washed out during the process of isolation; but it is difficult to believe that these could be other than quartz and small amounts of non-crystalline free silica. The resulting mixture of mica with a little kaolin and 1 per cent. of quartz constitutes "fine fractions" and is probably a sufficiently pure form of hydromica to furnish a reasonable test of pathogenicity for the micaceous element in shale. X-ray diffraction analysis has revealed no contaminant which could be held responsible for the non-pathogenicity of the mica, through a depressant action on its activity; and the mica (sericite) of the South Wales shales must therefore be regarded as non-fibrogenic.

It was not possible to isolate kaolin from the shale of South Wales as a separate fraction. But the mixtures constituting the extraneous mineral matter from coal have a high proportion of kaolin in their siliceous part, and in one of them—A2 LP (Table XV)—kaolin constitutes 67 per cent. of the silicate fraction. Experiments with these dusts do not, of course, constitute an adequate test of the kaolin fraction of shale, but it is worth noting that they all come within the negative group (p. 46).

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\* Bracketed dusts are from sources other than South Wales.



It must not be overlooked, however, that minute quantities of some other component of these shales—some constituent of an antidotal character, active in very small amount—may be responsible for all the negative results with shale, its fractions, and mixed dusts of which it is a constituent.

Dusts of either the positive or the negative group behave initially in the same way when introduced into the lung, namely by immobilizing the phagocytes. This effect is relative and varies in degree from one dust to another; it is evidently not merely the result of gravity. It seems likely that the cytoplasm of the koniophage undergoes some physical change as a consequence of being loaded with silica; the viscosity is, perhaps, increased, the surface tension altered.

From this point onwards in the reaction, the two groups of dusts diverge. In the negative group the koniophage persists *in statu quo*; it remains intact, relatively immobilized, yet detached and potentially free. In the case of positive dusts, the cell is soon caught up by newly formed connective tissue fibrils and secured in a fibrillar snare.

With the positive dusts there is evidence of acute inflammatory change at an early stage, whereas the negative dusts typically do not show this concomitant.

On the basis of these findings, the mechanism of fibrogenesis could be explained as follows: Particulate silica undergoes a slight degree of dissolution when embedded in the cytoplasm of a phagocyte, liberating enough silicic acid to increase viscosity and alter the surface tension of the cytoplasm, thus decreasing movement while, at the same time, depressing metabolism, inhibiting autolytic enzymes and causing an artificial preservation of the cell. Unless something happens to break this paralysing yet preservative effect, the cell seems to remain *in statu quo* indefinitely, and the silicic acid is retained within the cell membranes, failing to come in contact with connective tissue elements and hence failing to stimulate fibrosis.

If, however, an acute inflammatory reaction supervenes, whether from fortuitous causes or as a result of silicic acid escaping from the koniophages, at first perhaps from only a few of them, then autolytic enzymes may be liberated in force and silicic acid also, thereby creating the necessary stimulus for fibrogenesis.

This theory places major importance upon relative solubility of the silica and, in doing so, conforms with the most widely accepted modern views as to the *modus operandi* of silica, but with this difference that, according to our findings, much depends upon the relative integrity of the koniophage; and that integrity, in turn, is vulnerable to extraneous influences like concomitant inflammation which may sometimes be due to fortuitous irritants or complications not amenable to rigid control in an experimental procedure such as we have employed.

The apparent solubility of silica is depressed by certain substances, notably by aluminium (cf. King, pp. 69-94 below) but also by shale and certain silicates.

Thus the positive dusts listed may in some cases owe their fibrogenic properties to chance, e.g. concomitant inflammation of unknown aetiology in the early stages of the experiments. Also, the negative group may, in some cases, owe their inactivity to chance in the form of extrinsic or intrinsic agents which depress their solubility; but—judging by the control experiments, group (a)—the fortuitous influence affects only a minority of animals in any given experiment. In the case of group (c), for example, shale possesses an intrinsic depressant effect on silica solubility, probably sufficiently potent to nullify the effect of any fortuitous irritants which might facilitate disintegration of the koniophages. The same dust when mixed with pure quartz forestalls fibrogenesis almost certainly by reducing the solubility of the quartz.

In the case of pure quartz, the sporadic negative results are more difficult to explain. Are the positive results with quartz due to a fortuitous adjuvant (e.g. acute inflammation) and are the negative results due to the fortuitous inclusion of some depressant of silica-solubility? Probably quartz is capable of producing inflammatory change by the liberation of silicic acid but does not always do so; it may well be that fortuitous irritants sometimes assist in instituting the inflammatory process which is a necessary antecedent for the carnification type of organization characteristic of the experimental silica fibrosis. But the alternative, namely the fortuitous intrusion of an antidotal substance, is also a possibility which cannot be excluded in the case of negative reactions. Indeed, it is possible that both adjuvants and antidotes are sometimes at work, and that the ultimate result depends upon the outcome of the reaction between them.

It is also possible that natural defence mechanisms in the animal lung provide their own antidote: for example, they may attempt to coat quartz particles with a film of lipoid material which retards or prevents the liberation of silicic acid. We know of several extraneous substances which depress silica-solubility (aluminium, iron, shale, several silicates), and there may be many others. It is difficult to be sure of excluding all fortuitous antidotes from the inoculum, and impossible to rule out endogenous antidotes.

Those air-borne dusts of group (f), Table XVI, which were fibrogenic had somewhat more siliceous matter in them than the negative ones, but this siliceous matter was derived for the most part from the very strata (the clods or shales) which showed so little fibrogenesis, and its composition will be seen to be very similar to that of the shales and the extraneous mineral matter likewise falling within the negative group.

Although the kaolin was not very fibrogenic (grade 2 or possibly 3) it was definitely in the active class. Whether its activity can be attributed to the 8 per cent. of hydrated free silica it contains is not clear. Kettle (1934b) found another sample of kaolin, likewise containing a little free silica and mica, to be fibrogenic in the presence of dead tubercle bacilli (B.C.G.), but it was relatively inert in their absence, and Middleton (1936) inclines to the belief that kaolin as breathed by the workers in the China-clay industry is not to be regarded as a dangerous dust. We have already noted the absence of fibrogenic response to those mixed dusts from South Wales containing the highest proportion of kaolin among their siliceous components (p. 46).

The Ogofau sericite, or hydromuscovite, as it is better called (Brammall *et al.*, 1937) was the most active dust tested next to quartz, and for this unexpected result no final explanation can yet be given. From the description given by Brammall *et al.*, it does not appear to differ greatly from the South Wales mica or sericite (fine fractions) studied by Hicks and Nagelschmidt (p. 164). The entirely different tissue reaction to this mica must be accepted, then, as a curious anomaly, which it is hoped will be elucidated by further experiment. The possible effect of acid treatment of the specimen on its pathogenicity is referred to on p. 87, and is being tested in other animal investigations. In the meantime, these several instances of apparent contradiction in our experiments need to be emphasized, since they demonstrate again what Kettle (1934c, 1935) often pointed out, namely that the response of a living organism to the presence of foreign matter in its tissues is a highly complex affair, and not to be regarded in terms of a simple test-tube reaction. There are many more factors at work than the physical and chemical attributes of the dust, and these may modify, or even condition, the type of reaction set up. Perhaps it is among the improperly understood biological factors that the explanation of our anomalies will finally be found.

The role of inflammation in the production of these experimental dust lesions has appeared to us uncertain and perplexing. Despite the high degree of uniformity of results in any single experiment, we have not been able to

convince ourselves that we are familiar with all the factors which go to produce the initial inflammatory lesion. The carnification type of organization is determined by antecedent inflammation, which is not always due to the irritant effect of the dust itself. The simple solubility theory of silicosis, while undoubtedly the most plausible to date, is insufficient to account for all our results. While in the main the more soluble silica dusts are most fibrogenic, this rule, as already noted by Gardner (1938b), does not always hold. Quartz, the most soluble dust we have used, is the most fibrogenic, shale is the least soluble and the least fibrogenic and, moreover, shale depresses both the solubility and the pathogenicity of quartz. But while quartz is fibrogenic in all animals, it is not invariably fibrogenic in all parts of the lung where it comes to rest. It is a fact that quartz may, and often does, settle in considerable quantities in the lung without provoking inflammation, carnification or fibrosis; and this leads us to think that there must then be lacking some factor or condition which is present whenever inflammation does set in and which is obligatory to fibrogenesis.

Does silica have to dissolve in order to produce inflammation? Or does inflammation create a local condition which accelerates the dissolution of silica? If the latter is true, what causes the initial inflammatory reaction? When we have the answers to these questions, we may reach a better understanding of the manner in which a tissue reacts to the presence of a siliceous dust.

### 8. SUMMARY

For the purpose of this final summary, the dusts are regrouped according to composition.

*Pure coal.*—All dusts consisting of 90 per cent. or more of coal, whether anthracite, bituminous or steam coal, whether sterilized or contaminated with living soil bacteria, were uniformly attended by minimal fibrosis. The dust deposits tended to become thinned out and scattered as time went on, lodging mainly in the interstitial tissues, where they produced nothing more than the simplest type of foreign-body reaction with fibrosis only of grade 1. There was evidence of concomitant emphysema, and presumably some decrease in vital capacity. The animals lived from three to six months less than normal controls. The histological picture as a whole simulates the dust reticulation of coalminers, though it is somewhat more uneven in distribution in the individual lung.

*Dusts of mixed composition.*—The majority of coal dusts with a content of siliceous material of more than 12½ per cent. produced something more than a simple foreign-body reaction. The reaction changed to a type of organization similar to carnification. The distribution tended to become nodular or confluent, and the density of reticulin fibrosis rose to grades 2 and 3. The silica in these dusts was mainly in the form of minerals of the mica and kaolin groups, and the amount of quartz was very small—even negligible in some cases—thus affording evidence that some silicates may be fibrogenic. But in other experiments (groups (b), (c), (g) and (h)), silicates comprising clod strata (shale) not only failed to produce fibrosis above a bare minimum but even appeared to inhibit the fibrogenic properties of quartz. Thus there is no uniformity in the pathogenicity of silicates as a whole; on the contrary, there seems to be a very considerable spread. With the exception of the two samples of extraneous silicates in group (h), Table XVIII, and the more fibrogenic members of group (f), Table XVI, in which silicates are at least suspect, all other samples of silicates fall within the negative group of dusts as indicated on p. 62.

*Stone dusts.*—These dusts, including samples from all the principal rock strata of one high incidence anthracite mine and shale strata of two other

mines, produced widely different reactions. But one striking feature was evident, namely that samples containing appreciable proportions of shale tended to fall into the negative group, with the exception of a few air-borne dusts of group (f), Table XVI, which, though apparently containing appreciable moieties of shale, produced a little more than minimal fibrosis. These air-borne dusts, however, are chiefly composed of coal and contain a wide variety of siliceous minerals, thus complicating the picture, so that it becomes impossible to say which constituents account for the fibrosis. Samples of the sandstone stratum of mine A3, containing 71 per cent. of quartz, were strongly fibrogenic and stood in marked contrast to samples of other rock strata (comprising clods and clift) chiefly composed of shale, none of which approached it in fibrogenic properties.

*Isolated fractions.*—Isolated fractions of mine dusts, comprising 5 samples, 4 of which were more or less pure mica (or sericite), showed a wide discrepancy in results. Three of these, each from a different mine, were designated as "fine fractions" and represented what is known as South Wales sericite in almost pure form. All 3 samples showed a uniform reaction, characterized by the simplest form of foreign-body reaction with an absolute minimum of reticular fibrosis even after 12 months—the slightest by far of all the late stage reactions in the whole series—indicating a very low pathogenicity. The other sample of mica (hydromuscovite or sericite) was from a source other than South Wales, and was employed as a control. It produced a heavy nodular reticulon fibrosis of grade 4 intensity, practically indistinguishable from the reaction to pure quartz. This sharp discrepancy provides the most striking anomaly in the entire series of experiments and is, as yet, unexplained. Exact chemical and mineralogical analyses may bring to light some difference in composition to correlate with the difference in tissue reaction; or the explanation may lie in a previous treatment with acid to which this sample had been subjected (cf. p. 87).

The fifth sample in this group (h) was kaolin, also from a source other than South Wales; it was thought to be pure kaolin, but turned out to contain no less than 8 per cent. of free silica. The tissue reaction was of grade 3 reticulon fibrosis. Because of the free silica content, the results cannot be regarded as representative of kaolin. Accordingly, the experiment is merely recorded and no inference is drawn from it.

*Mineral matter recovered from human lungs.*—One sample was derived from a case of silicosis, the other from a case of dust reticulation, both from South Wales; they produced grade 4 and grade 1 fibrosis, respectively.

*Artificial mixtures of pure quartz with various mine dusts.*—Experiments with these mixtures showed that the pathogenicity of quartz tended to be reduced by clean coal and practically abolished by shale. Mixtures of impure coal and quartz produced diffuse and massive indurations, closely resembling the forms of major consolidation found in coalminers (Belt and Ferris, 1942).

The initial impact of silica appears to fall upon the phagocyte, reducing its mobility. So long as the phagocyte remains intact, the silica is evidently prevented from extending its effect to the connective tissue. An inflammatory reaction may help to bring about disintegration of the phagocyte, thus freeing the silica to exert its fibrogenic effect.

The incidence of acute inflammatory reaction following the injection of dusts (as determined by examining 2 animals from each experiment between the third and sixth days) was closely parallel with the higher grades of fibrogenesis. It is uncertain that this initial inflammation was directly attributable to the dust in all cases. Allowance has to be made for complicating factors incidental to the technique, though the general uniformity of results in

individual experiments tends to minimize the probability of fallacies on this score.

The results show that the tissue reaction to silica is not always a simple matter of impact of silica on living cells but entails an interplay of adjuvant and antidotal factors; some of these, like the adjuvant effect of tubercle in human silicosis, and the antidotal effect of aluminium, are known, while others, especially in experimental pneumokoniosis, are unknown and not amenable to rigid control in animal dusting experiments.

In other words, the tissue reaction to silica is a complex biological phenomenon which cannot be subjected to precise control. Likewise, histological methods of assessing results of dusting experiments are necessarily lacking in precision, though serial sections, micro-incineration and the quantitative estimation of reticulin tend to reduce the margin of error. The high proportion of uniformly positive results in the control experiments with quartz, however, indicates that fortuitous factors affect only a minority of the injected dusts and therefore do not invalidate the experiments.

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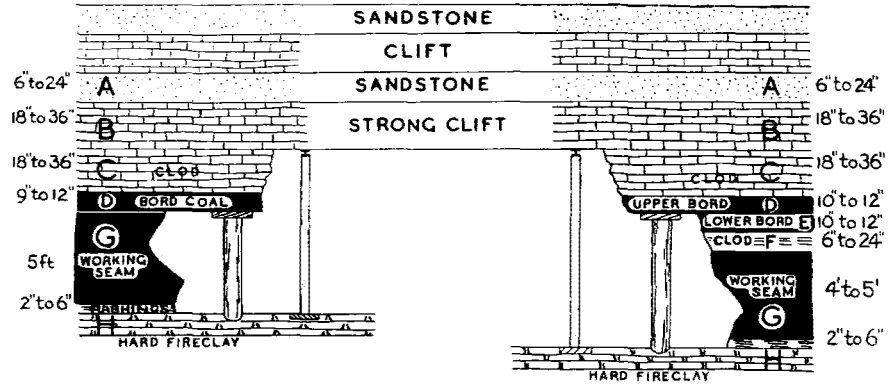
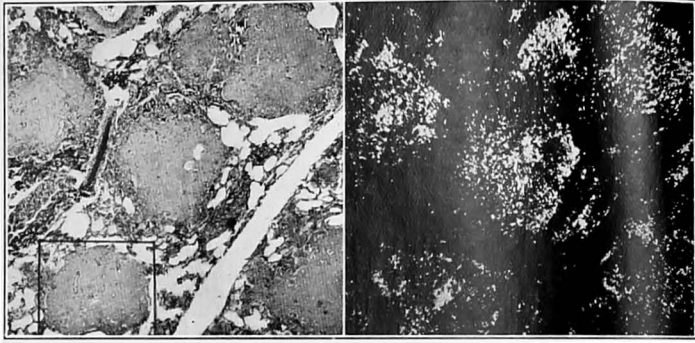


FIG. 1.—Diagrammatic representation of geological strata of mine A3 (from a drawing by Mr. D. Jeffreys). This is a typical "high-incidence" anthracite mine. Powdered samples from each of the principal strata were tested in animals. The results showed that stratum A, sandstone, was more pathogenic than the others. For chemical composition and particle size of each sample, see Table XII, p. 45.



\* FIG. 2.—*Dust*: Pure quartz, Table XI. *Duration*: 7 months. *Magnification*: 22. *Area*: Cluster of nodules. *Serial sections*: Left: H and E. Right: HCl-insoluble ash, dark field. *Comment*: See Fig. 3 for higher magnification of marked area.

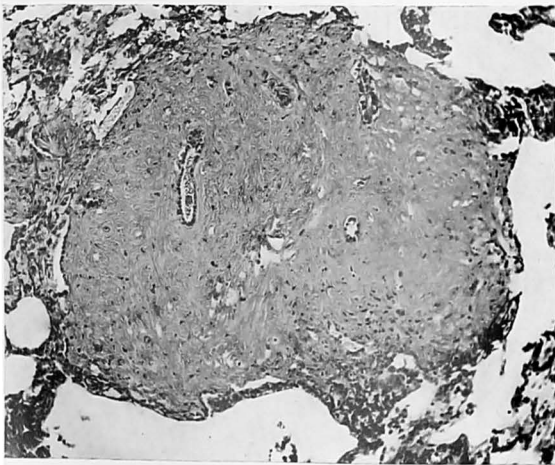


FIG. 3.—*Dust*: pure quartz, Table XI. *Duration*: 7 months. *Magnification*: 95. *Area*: marked area (single nodule) from Fig. 2. *Serial sections* - part 1, H. and E. above (see opposite page for parts 2 and 3, viz. HCl-insoluble ash and reticulin stain). *Lesion*: Nodular dust-reticulation. *Organization*: Carnification type. *Distribution*: Nodular. *Fibrosis*: Maximal, grade 4. *Comment*: Neither quartz nor fibres visible in H. & E. section. Small lacunae in nodule lined by cuboidal epithelium represent beginning recanalization or reopening of air sacs.



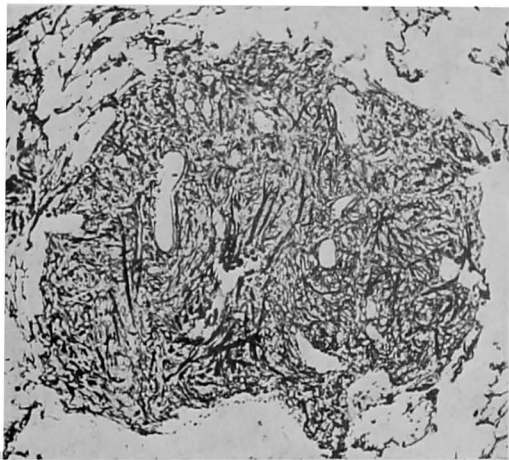
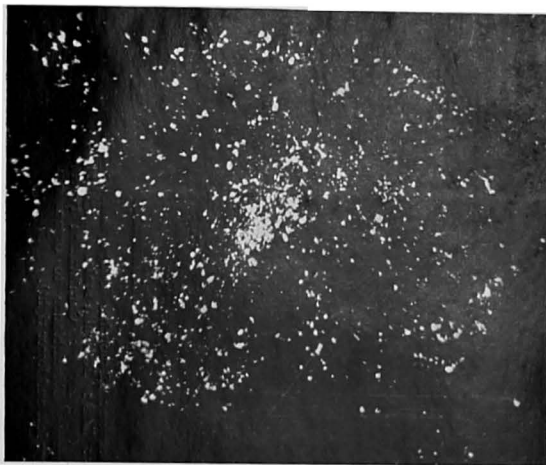


FIG. 3 (*continued*).—Parts 2 & 3.—**Upper**: HCl-insoluble ash, dark field. **Lower**: Reticulin stain. See also Figs. 29 and 30 for quartz reaction at 17 and 18 months. (Fig. 3 is reproduced, by kind permission, from *J. Path. Bact.*, 1940, **51**, Plate XXXVI.)

[*Note*.—The use of a hand-lens is advised for examining the reproductions of sections stained for reticulin, Figs. 3 (above), 4, 6, 7, 10, 12, 13, 14, 15, and 17-29.]

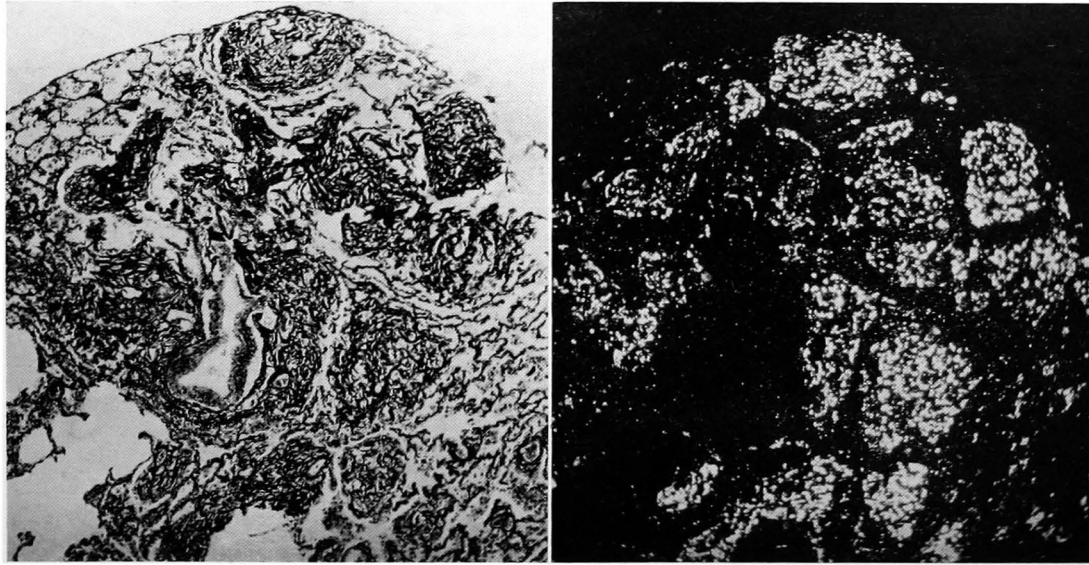


FIG. 4.—*Dust*: Stratum A (sandstone) from mine A3 (see Fig. 1). *Duration*: 12 months. *Magnification* 75. *Area*: Between terminal bronchioles and pleura. *Serial sections*: **Left**: Reticulin stain. **Right**: HCl-insoluble ash, dark field. *Lesion*: Nodular dust-reticulosis. *Organization*: Carnification type. *Distribution*: Nodular, intra-alveolar. *Fibrosis*: Grade 4. *Comment*: Resemblance to organizing pneumonia well marked. Some silica still in loose-lying phagocytes (upper left and lower right).

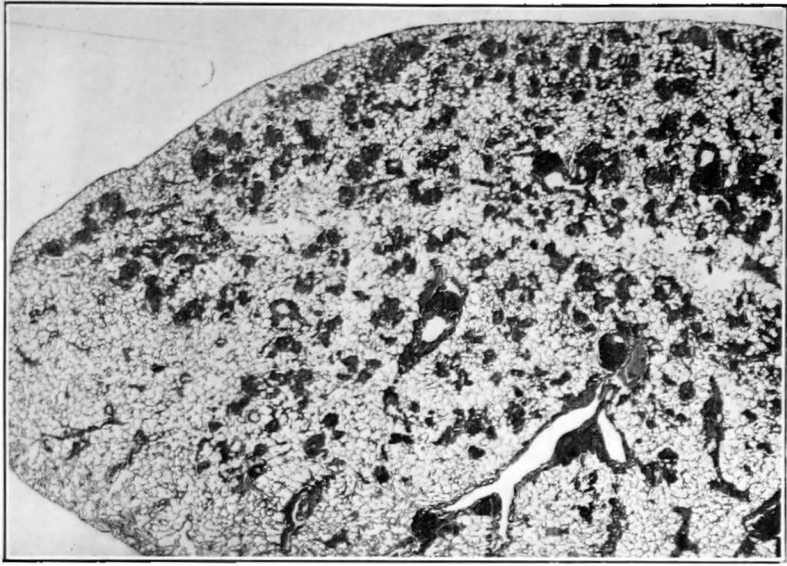


FIG. 5.—*Dust*: Stratum B clift (shale) from mine A3 (see Fig. 1, and p. 44). *Duration*: 8 months. *Magnification*: 7 (ca). *Area*: Apical part of left upper lobe. *Stain*: H. and E. *Comment*: An even distribution of dust like that pictured here was obtained in the majority of experiments. See Fig. 6 for higher magnification.

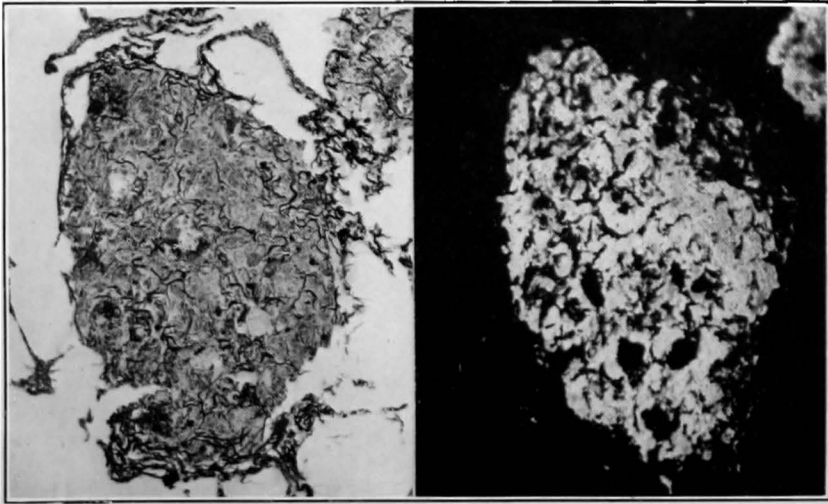
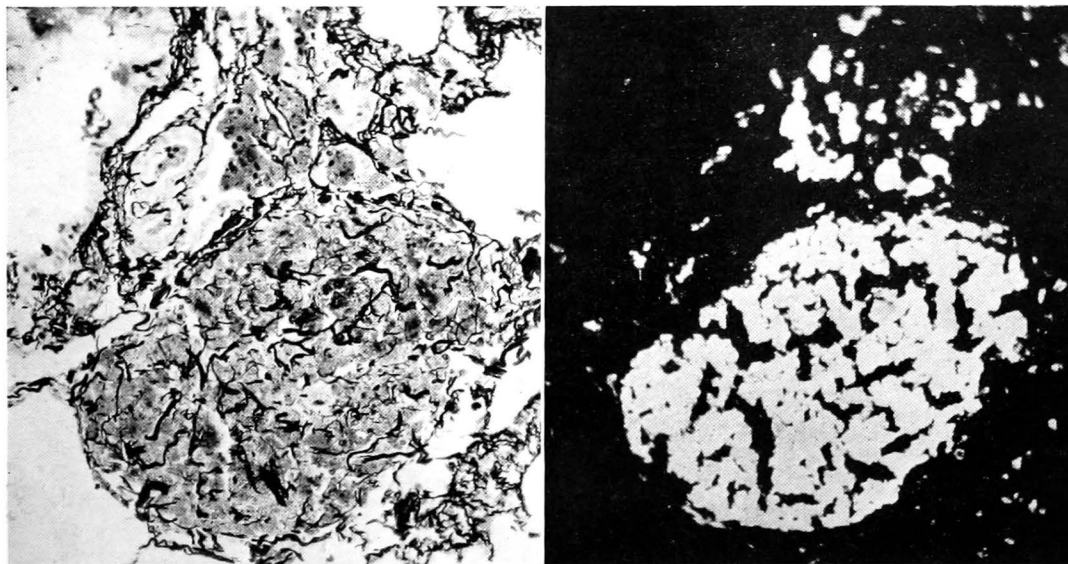


FIG. 6.—*Dust*: Stratum B clift (shale) from mine A3 (see Fig. 1, and p. 44). *Duration*: 8 months. *Magnification*: 110. *Area*: Single nodule from Fig. 5. *Serial sections*: **Left**: Reticulin stain. **Right**: HCl-insoluble ash, dark field. *Lesion*: Nodular dust-reticulosis. *Organization*: Foreign body type with few giant cells. *Distribution*: Nodular, intra-alveolar. *Fibrosis*: Minimal, grade 1. *Comment*: Paler areas in nodule are large particles of crystalline mineral, probably quartz.



**FIG. 7.**—*Dust* : Stratum C (clod) from mine A3 (see Fig. 1, and p. 46). *Duration* : 7 months. *Magnification* : 150. *Area* : Single nodule. *Serial sections* : **Left** : Reticulin stain. **Right** : HCl-insoluble ash, dark field. *Lesion* : Nodular dust-reticulinos. *Organization* : Mainly foreign-body type with some giant cells. *Distribution* : Nodular, intra-alveolar. *Fibrosis* : Grade 1.

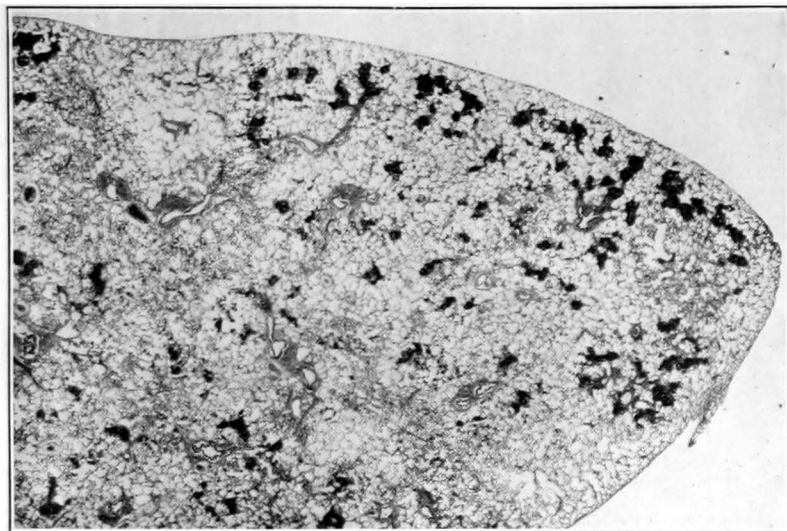


FIG. 8.—*Dust* : Stratum D (Bord Coal) of mine A3 (see Fig. 1, and p. 46). *Duration* : 8 months. *Magnification* : 7. *Area* : Apical part of left upper lobe. *Stain* : H. and E. *Comment* : Sometimes the dust tends to lodge in sub-pleural zone as illustrated here. The thin visceral pleura of the rodent lung however permits of no appreciable deposits within the membrane itself. See Fig. 9 for higher magnification.

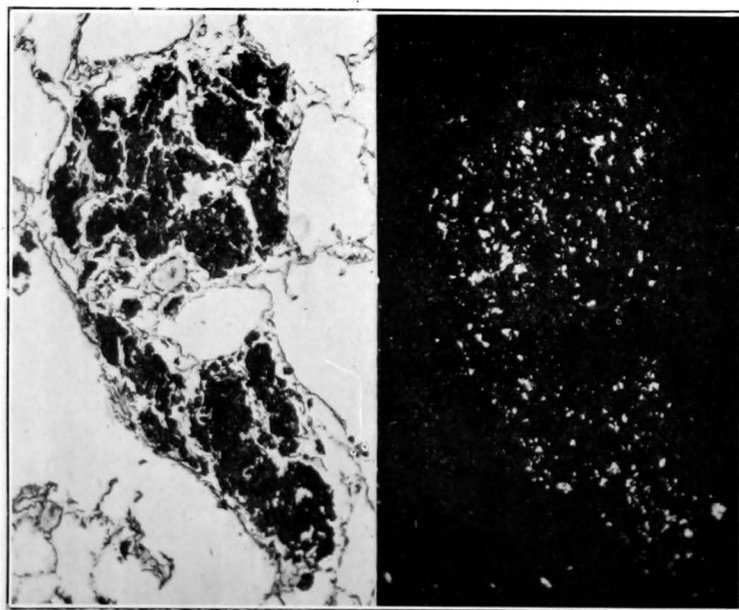


FIG. 9.—*Dust* : Stratum D (Bord Coal) of mine A3 (see Fig. 1, and p. 46). *Duration* : 8 months. *Magnification* : 100. *Area* : Single nodule from Fig. 8. *Serial sections* : **Left** : Reticulin stain. **Right** : HCl-insoluble ash, dark field. *Lesion* : Nodular dust-reticulinos. *Organization* : Foreign-body type with giant cells. *Distribution* : Nodular, ? intra-alveolar. *Fibrosis* : Minimal, grade I. *Comment* : Difficult to say whether this deposit is intra-alveolar or interstitial. Irregularity in the size of air-sacs indicates emphysema.

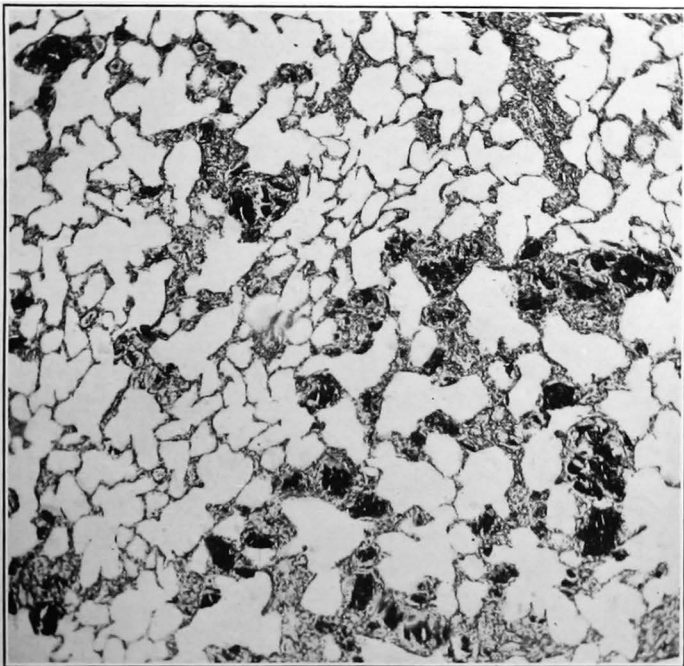


FIG. 10.—*Dust* : Low ash coal fraction—clean coal from mine A3 (anthracite). *Duration* : 8 months. *Magnification* : 60. *Area* : mid-part of lung. *Stain* : Reticulin. *Lesion* : Dust-reticulosis. *Organization* : Foreign-body type with giant cells. *Distribution* : Scattered; mainly interstitial. *Fibrosis* : Grade 1. *Comment* : Coal dust tends to clump, giving impression of large particles but actually particles average about  $1\mu$  (see Table XIV). Insufficient ash to photograph.

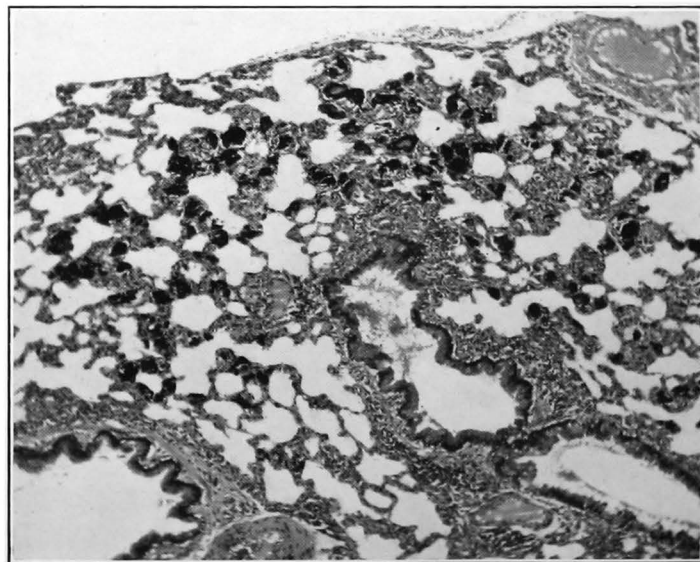


FIG. 11.—*Dust* : Low ash coal fraction—clean coal from mine S4 (steam coal). *Duration* : 8 months. *Magnification* : 60. *Area* : Between terminal bronchioles and pleura. *Stain* : H. and E. *Lesion* : Dust-reticulosis. *Organization* : Foreign-body type with giant cells. *Distribution* : Scattered; interstitial. *Fibrosis* : Grade 1. *Comment* : Reaction simulates dust-reticulation (dust-reticulosis, see p. 42) of coalminers, the more so because of concomitant emphysema. Insufficient ash to photograph.

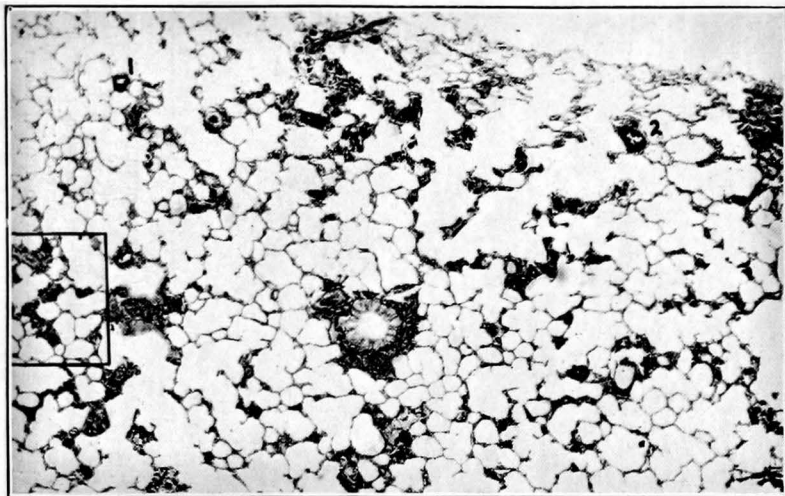


FIG. 12.—*Dust* : Low ash coal fraction—clean coal from mine B2 (bituminous). *Duration* : 17 months. *Magnification* : 30. *Area* : Between terminal bronchioles and pleura. *Stain* : Reticulin. *Lesion* : Dust-reticulosis and emphysema. *Comment* : Emphysema well marked. Note isolated giant cells 1 and 2 containing dense rings of coal particles. See Fig. 13 for higher magnification of marked area.

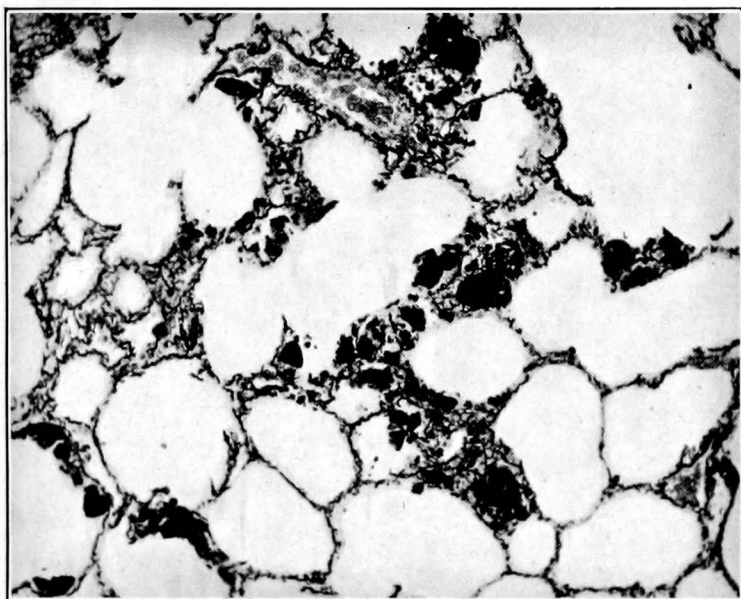


FIG. 13.—*Dust* : Low ash coal fraction—clean coal from mine B2 (bituminous). *Duration* : 17 months. *Magnification* : 145. *Area* : Marked area in Fig. 12. *Stain* : Reticulin. *Lesion* : Dust-reticulosis. *Organization* : Foreign-body type with few giant cells. *Distribution* : Scattered ; mainly interstitial. *Fibrosis* : Grade I. *Comment* : At 17 months the dust is thinly distributed along the septa ; original nodular distribution completely superseded. Practically no ash remains after incineration.

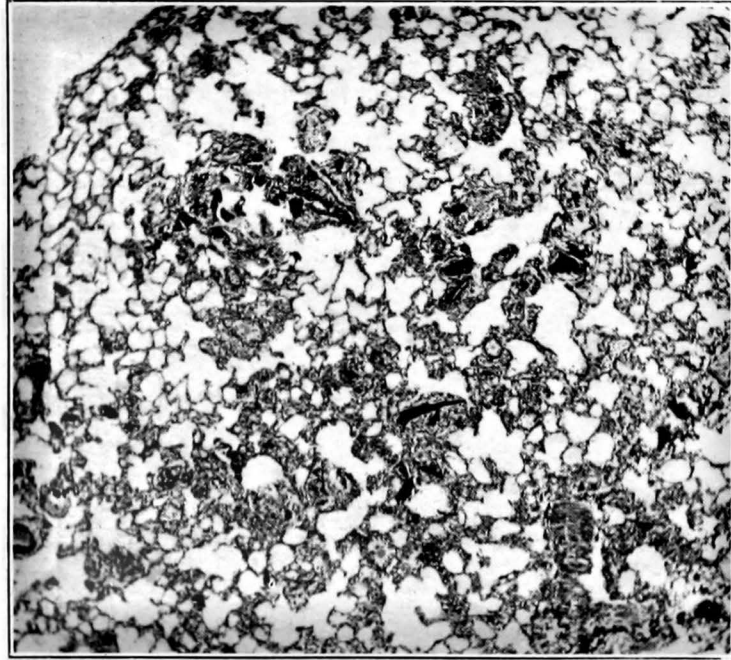


FIG. 14.—*Dust*: High ash coal fraction (extraneous mineral matter) from coal seam of mine A3 (anthracite). *Duration*: 8 months. *Magnification*: 65. *Area*: Between terminal bronchioles and pleura. *Stain*: Reticulin. *Lesion*: Dust-reticulosis and emphysema. *Organization*: Foreign-body type with giant cells. *Distribution*: Scattered; interstitial. *Fibrosis*: Grade 1. *Comment*: Practically the same reaction as to clean coal (cf. Figs. 10 to 13) though this dust is 15 per cent. siliceous (see Table XV). Dust is clumped; individual particles are much smaller.

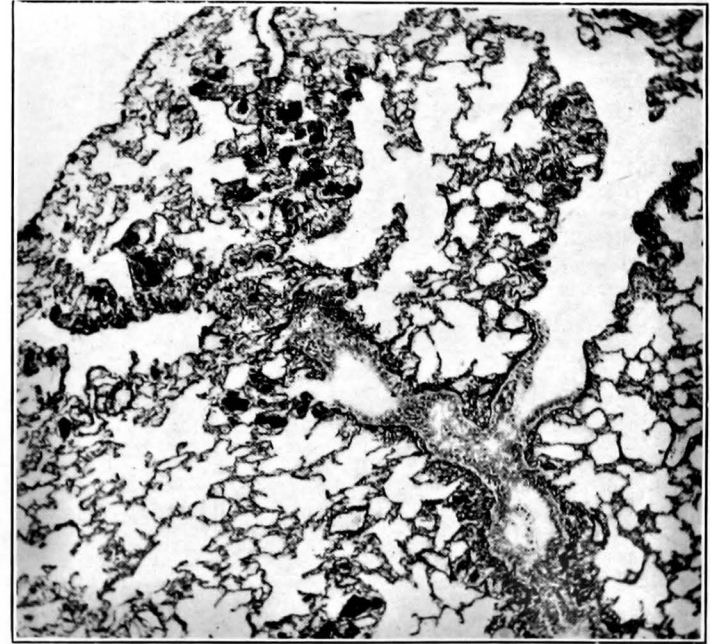


FIG. 15.—*Dust*: High ash coal fraction (extraneous mineral matter) from coal seam of mine S4 (steam coal). *Duration*: 8 months. *Magnification*: 60. *Area*: Between terminal bronchioles and pleura. *Stain*: Reticulin. *Lesion*: Dust-reticulosis. *Organization*: Foreign-body type with giant cells. *Distribution*: Scattered; interstitial. *Fibrosis*: Grade 1. *Comment*: Reaction similar to that of clean coal (cf. Figs. 10 to 13).



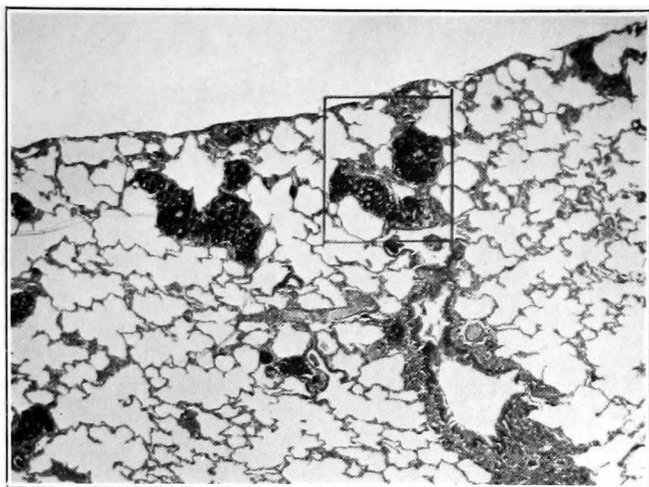


FIG. 16.—*Dust*: Airborne "I" from mine A3 (anthracite). *Duration*: 12 months. *Magnification*: 37. *Area*: Between terminal bronchiole and pleura. *Stain*: H. and E. *Lesion*: Nodular dust-reticulosis. *Comment*: Coal is usually more scattered, less nodular than this at 12 months; possible reason for persisting nodules is large proportion of giant cells. See Fig. 17 for higher magnification of marked area.

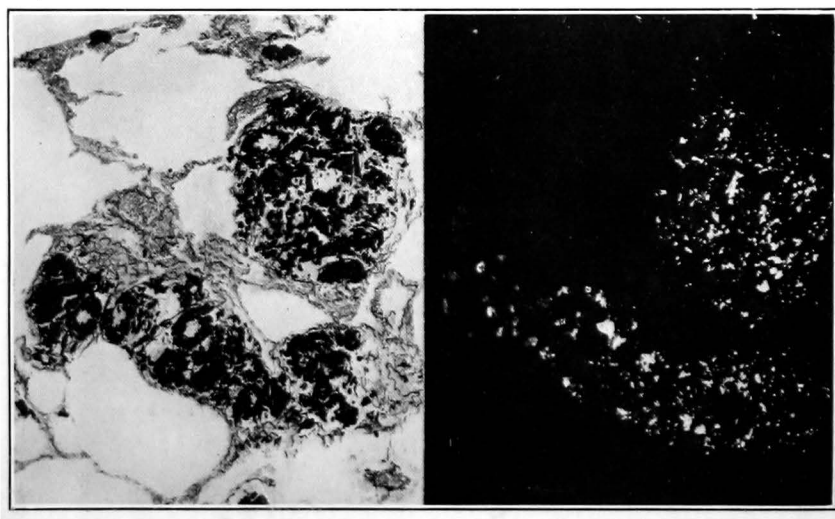


FIG. 17.—*Dust*: Airborne "I" from mine A3 (anthracite). *Duration*: 12 months. *Magnification*: 115. *Area*: Composite nodule from Fig. 16. *Serial sections*: **Left**: Reticulin stain. **Right**: HCl-insoluble ash, dark field. *Lesion*: Nodular dust-reticulosis. *Organization*: Foreign-body type with many giant cells. *Distribution*: Mainly nodular but with some scattering. *Fibrosis*: Minimal, grade 1. *Comment*: Nodules composed largely of dust-laden giant cells with particles frequently arranged in form of dense ring.

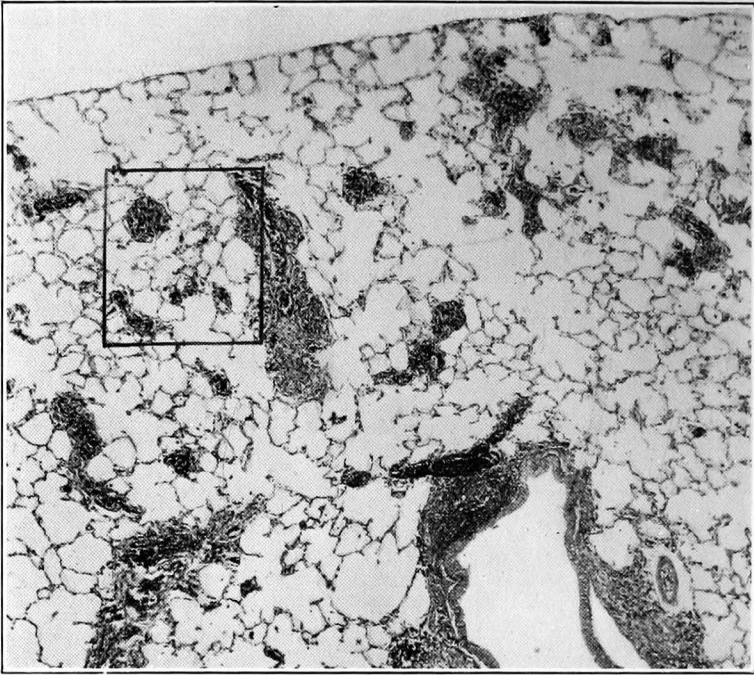


FIG. 18.—Dust: Airborne "E" from mine A3 (anthracite) (not to be confused with Stratum E). Duration: 12 months. Magnification: 40. Area: Between terminal bronchiole and pleura. Stain: Reticulin. Lesion: Dust-reticulosis and emphysema. Comment: Less clumping of particles here than in other experiments with coal dust, hence few, if any, giant cells. See Fig. 19 for higher magnification of marked area.

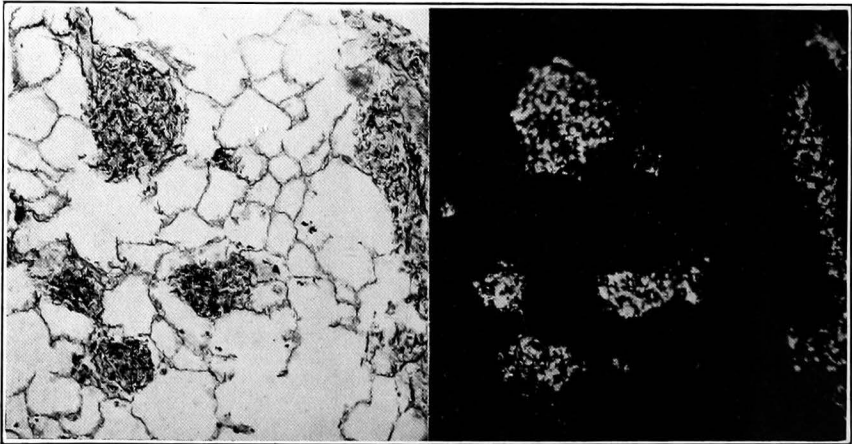


FIG. 19.—Dust: Airborne "E" from mine A3 (anthracite). Duration: 12 months. Magnification: 85. Area: Marked area in Fig. 18 (approx.). Serial sections—Left: Reticulin stain. Right: HCl-insoluble ash, dark field. Lesion: Finely nodular dust-reticulosis. Organization: Mainly carnification type. Distribution: Nodular and scattered; mainly interstitial. Fibrosis: Grade 2. Comment: Dust is 8 per cent. quartz and 30 per cent. silicate, which probably accounts for denser fibrillar reaction than in Figs. 16 and 17; but cf. Fig. 21.

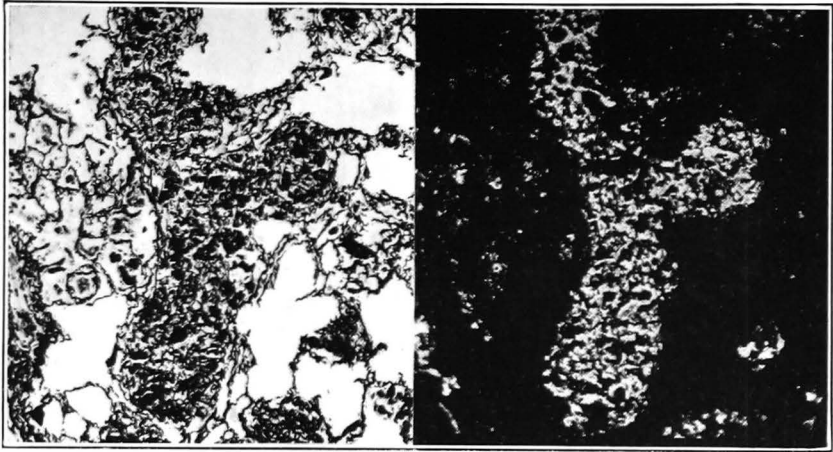


FIG. 20.—*Dust* : Airborne 1B1 from mine B3 (bituminous). *Duration* : 12 months. *Magnification* : 95. *Area* : Composite nodule. *Serial sections* : **Left** : Reticulin stain. **Right** : HCl-insoluble ash, dark field. *Lesion* : Nodular dust-reticulosis. *Organization* : Carnification type but with some foreign-body reaction. *Distribution* : Nodular and scattered; intra-alveolar and interstitial. *Fibrosis* : Grade 3. *Comment* : Dust contains 4 per cent. quartz and 16 per cent. mica. Initial inflammatory reaction was fairly intense. These factors probably account for denser fibrillar reaction than in case of other air-borne dusts. Note some silica still unorganized in loose-lying koniophages at left.

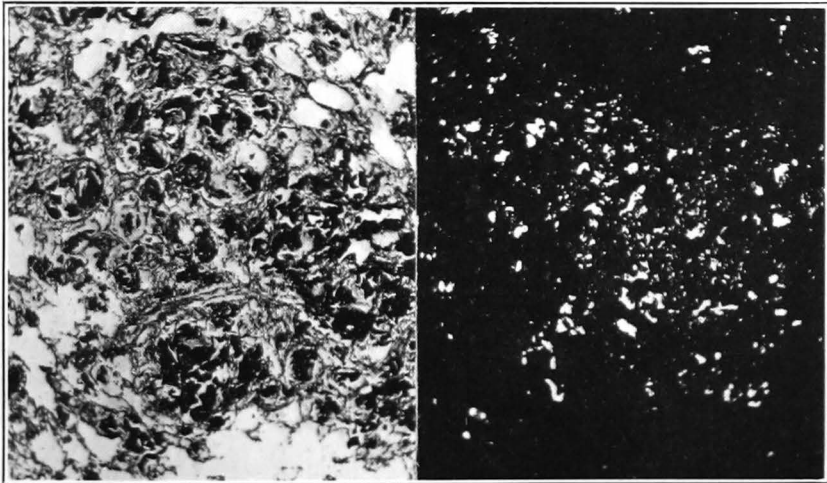


FIG. 21.—*Dust* : Screened Smalls (95 US 10) from mine A2 (anthracite). *Duration* : 8 months. *Magnification* : 75. *Area* : Composite nodule. *Serial sections* : **Left** : Reticulin stain. **Right** : HCl-insoluble ash, dark field. *Lesion* : Nodular dust-reticulosis. *Organization* : Carnification and foreign-body reaction with giant cells. *Distribution* : Coalescent nodulation; intra-alveolar and interstitial. *Fibrosis* : Grade 2. *Comment* : Initial inflammatory reaction was well marked, which probably accounts for confluence into large nodules and Grade 2 fibrosis, in spite of low silica-content of dust (see Table XVI) coal particles are rather heavily clumped.

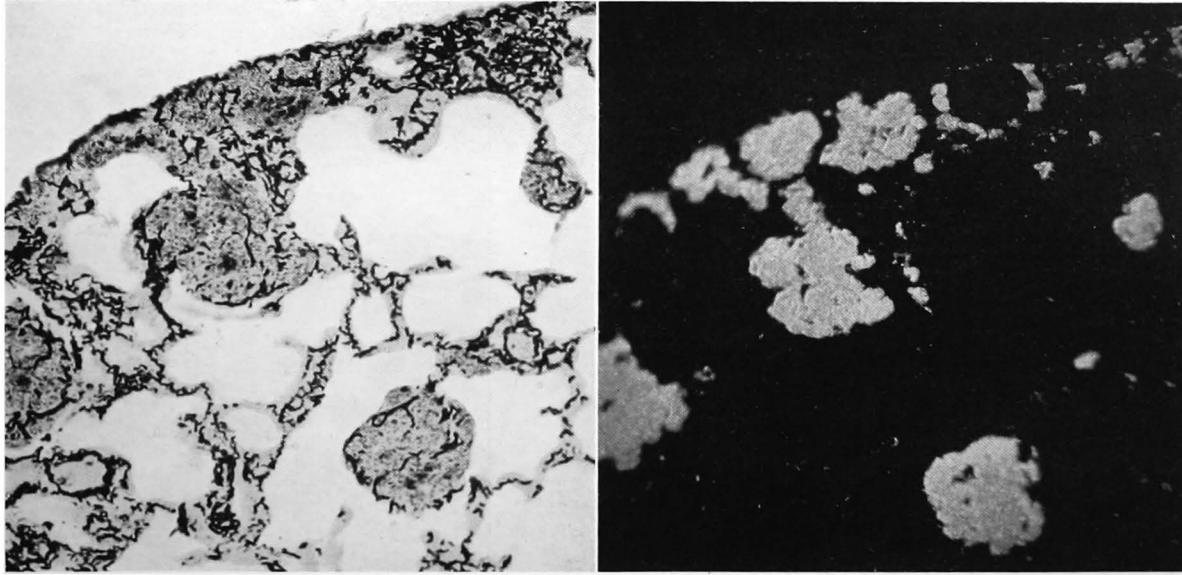


FIG. 22.—*Dust* : Fine fraction (mica, sericite) from mine A1. *Duration* : 12 months. *Magnification* : 165. *Area* : Near pleura. *Serial sections* : **Left** : Reticulin stain. **Right** : HCl-insoluble ash, dark field. *Lesion* : Finely nodular dust-reticulinosis. *Organization* : Simplest foreign-body type. *Distribution* : Nodular and scattered. Intra-alveolar and interstitial. *Fibrosis* : Minimal (practically nil but classified as Grade 1). *Comment* : Dust produces little or no reaction. This appearance was uniform throughout animals of all three experiments with "fine fractions."

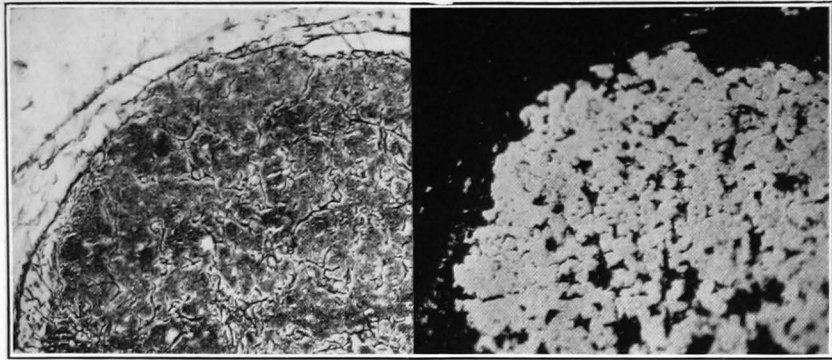


FIG. 23.—*Dust* : Fine fraction (mica, sericite) from mine S4. *Duration* : 12 months. *Magnification* : 105. *Area* : Sector of peri-bronchial gland. *Serial sections* : **Left** : Reticulin stain. **Right** : HCl-insoluble ash, dark field. *Lesion* : Confluent dust-reticulosis. *Organization* : Simplest foreign-body type. *Distribution* : Confluent. *Fibrosis* : Practically nil, Grade 1. *Comment* : Like "fine fractions" from other mines, this dust produces very little reaction. Compare quartz reaction in root gland, Fig. 29.

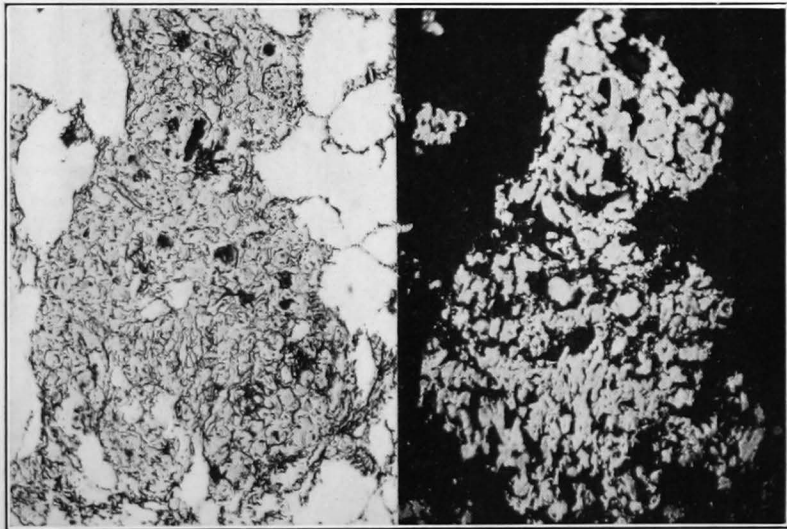


FIG. 24.—*Dust* : Kaolin (see Table XVIII). *Duration* : 8 months. *Magnification* : 80. *Area* : Single nodule. *Serial sections* : **Left** : Reticulin stain. **Right** : HCl-insoluble ash, dark field. *Lesion* : Nodular dust-reticulosis. *Organization* : Carnification type but with some giant cells phagocytosing large particles. *Distribution* : Nodular; intra-alveolar. *Fibrosis* : Grade 2 or 3 (?). *Comment* : Initial inflammatory reaction was well marked. Reaction like that of pure quartz, but with considerably less dense fibrillar reaction. Difficult to judge whether fibrosis belongs to Grade 2 or 3.

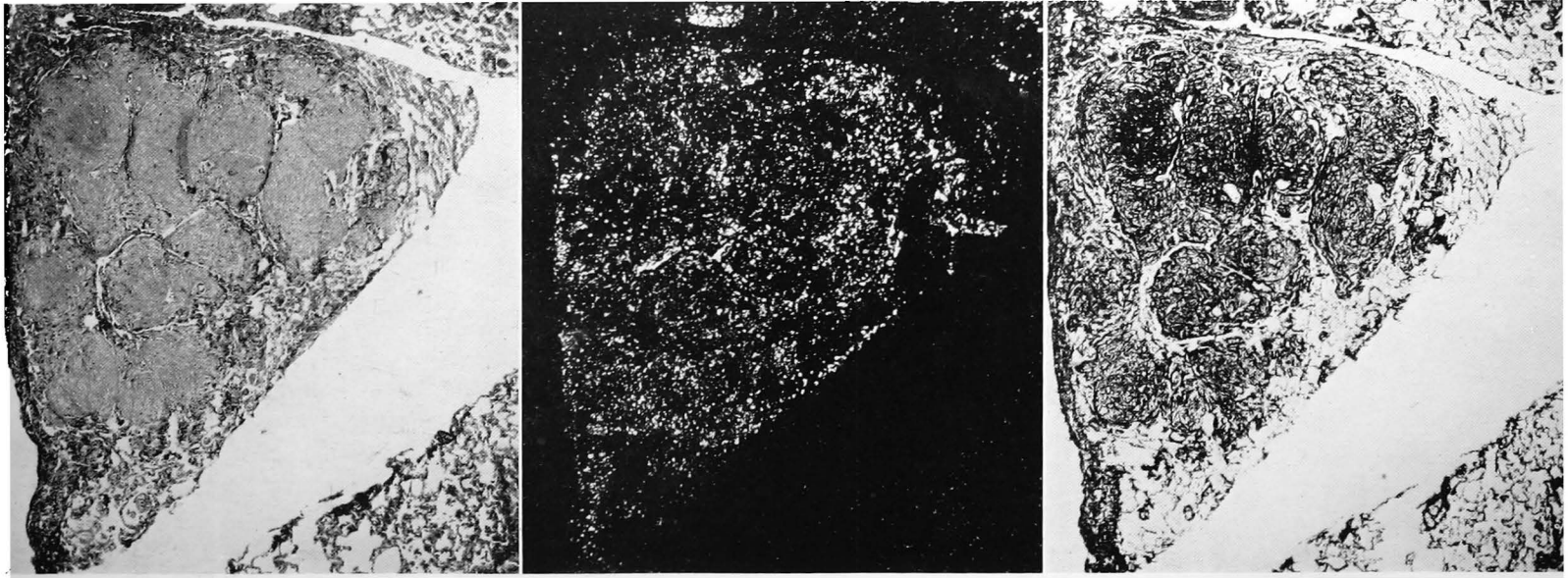


FIG. 25.—*Dust* : Ogofau sericite (control) ; from table XVIII. *Duration* : 12 months. *Magnification* : 35. *Area* : Group of confluent nodules. *Serial sections* : **Left** : H. and E. **Middle** : HCl-insoluble ash, dark field. **Right** : Reticulin stain. *Lesion* : Coalescent nodular dust-reticulosis. *Organization* : Carnification type. *Distribution* : Nodular ; intra-alveolar. *Fibrosis* : Grade 4. *Comment* : Reaction practically identical with that of pure quartz (cf. Figs. 2 and 3). Well-marked initial inflammatory reaction in this experiment possibly determined above result.

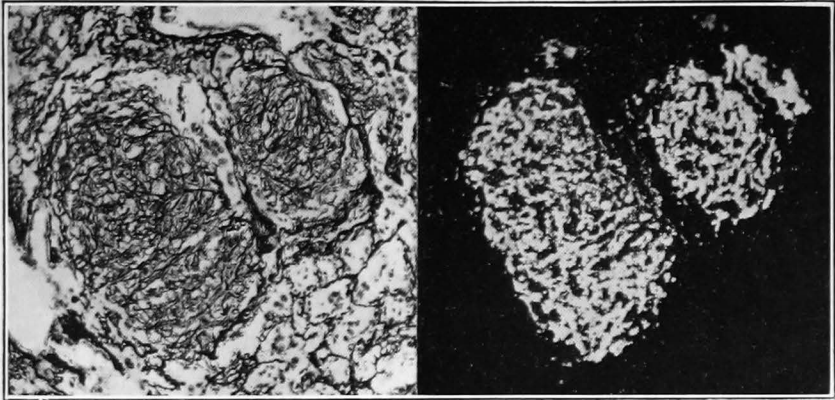


FIG. 26.—*Dust*: Residue from human lung, Specimen A (hard-header), case of silicosis. *Duration*: 12 months. *Magnification*: 100. *Area*: Single composite nodule. *Serial sections*: **Left**: Reticulin stain. **Right**: HCl-insoluble ash, dark field. *Lesion*: Nodular dust-reticulosis. *Organization*: Carnification type. *Distribution*: Finely nodular; intra-alveolar. *Fibrosis*: Grade 4. *Comment*: Compared with reaction to pure quartz, nodules are smaller, more discrete; fibrosis approximately equal in density. Initial inflammatory reaction quite well marked in two animals examined on fourth day after injection.

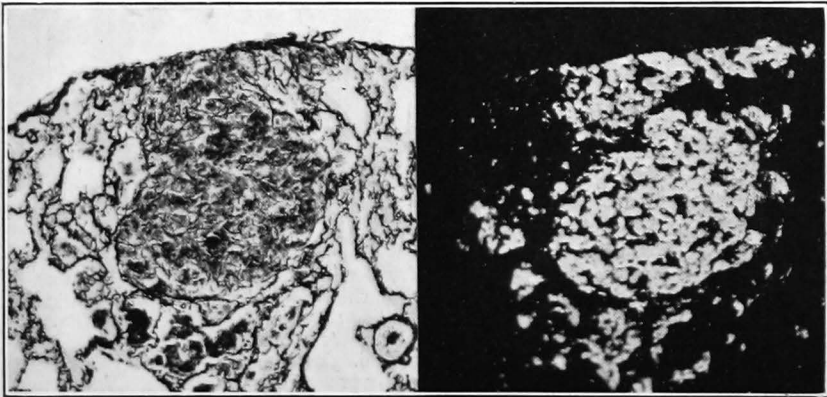


FIG. 27.—*Dust*: Residue from human lung, Specimen B (collier). Case of dust-reticulosis. *Duration*: 12 months. *Magnification*: 120. *Area*: Single subpleural nodule. *Serial sections*: **Left**: Reticulin stain. **Right**: HCl-insoluble ash, dark field. *Lesion*: Nodular dust-reticulosis. *Organization*: Simple foreign-body type. *Distribution*: Finely nodular and scattered; mainly intra-alveolar. *Fibrosis*: Minimal, Grade 1. *Comment*: In contrast to residue from a "hard-heading" coalminer (Fig. 26), this residue from a collier with simple dust-reticulation (reticulosis, see p. 42) produces very little reaction, though the dust has much the same composition (see Table XIX). Lack of fibrosis possibly result of absence of initial inflammatory reaction.



FIG. 28.—Dust: Quartz and clean coal from mine A3. Duration: 16 months. Magnification: 70. Area: Between terminal bronchiole and pleura. Stain: Reticulin. Lesion: Partial organization of quartz. Organization: Carnification type. Distribution: Diffuse, intra-alveolar. Fibrosis: Grade 4. Comment: Greater part of quartz still unorganized, lying in pale macrophages within alveoli. Small nodules of reticular tissue represent part of quartz which has been organized by process of carnification. This experiment illustrates that coal partially checks fibrogenic property of quartz, but other areas in this lung show extensive quartz fibrosis.



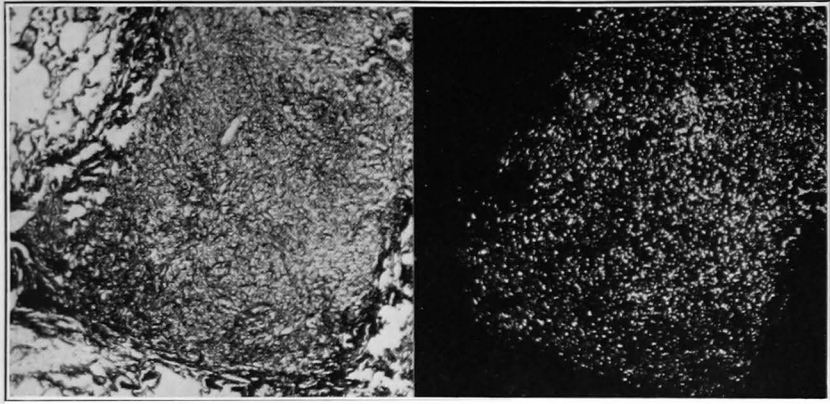


FIG. 29.—*Dust* : Mixture of pure quartz and clean coal from mine A1. *Duration* : 17 months. *Magnification* : 45. *Area* : Small root gland. *Serial sections* : **Left** : Reticulin stain. **Right** : HCl-insoluble ash, dark field. *Lesion* : Confluent dust-reticulosis. *Organization* : Post-inflammatory granulation tissue, equivalent of carnification in lung. *Distribution* : Confluent. *Fibrosis* : Grade 4. *Comment* : Admixture of coal dust failed to modify usual effect of quartz in lung as in root glands. Curiously enough, only quartz reached the glands, and practically no coal. This was possibly due to acute inflammatory change in early stage produced by quartz and not by coal and/or to smaller particle size of quartz.

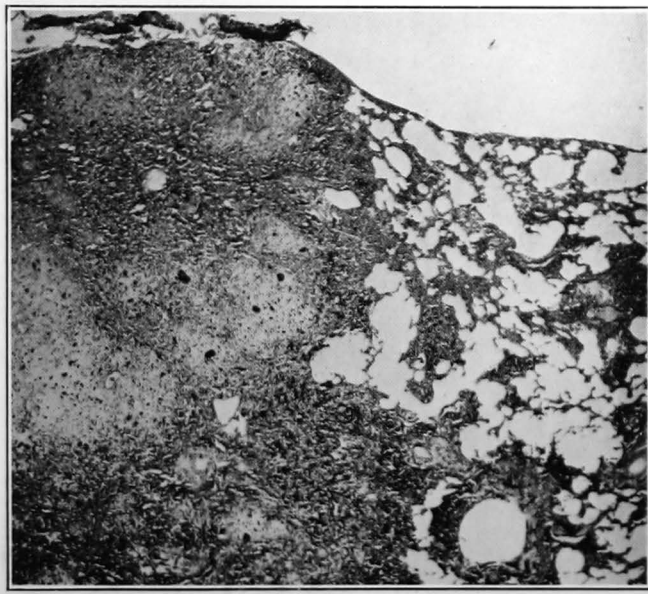


FIG. 30.—*Dust* : Quartz and airborne IBI from mine B3. *Duration* : 15 months. *Magnification* : 25. *Area* : Between terminal bronchiole and pleura. *Stain* : van Gieson. *Lesion* : Confluent dust-reticulosis (massive fibrosis). *Organization* : Carnification type. *Distribution* : Confluent and scattered. *Fibrosis* : Grade 4. *Comment* : Massive fibrosis simulating major consolidation of coal-miners' pneumokoniosis. Fibrous area liberally sprinkled with coal particles.

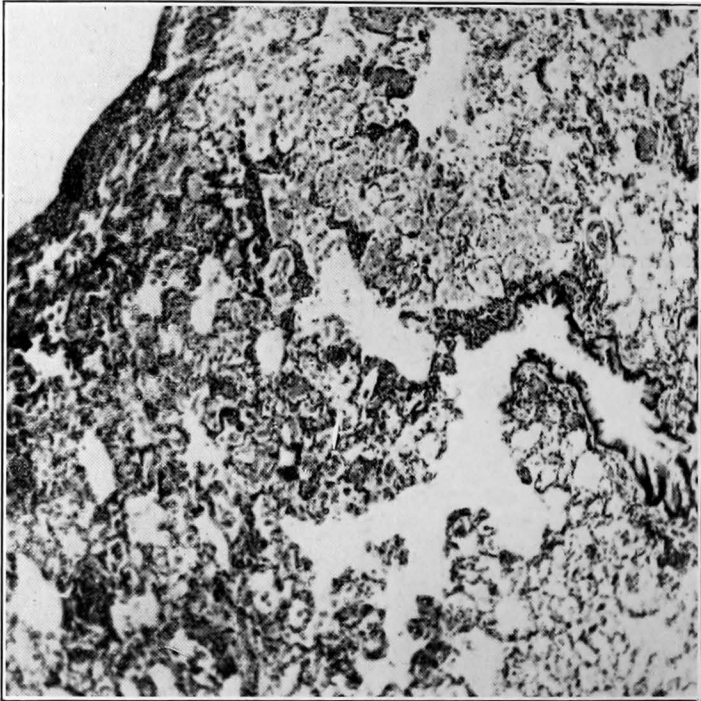


FIG. 31.—*Dust*: Quartz and Stratum C, clod (shale) from mine A3. *Duration*: 12 months. *Magnification*: 65. *Area*: Between terminal bronchiole and pleura. *Stain*: van Gieson. *Lesion*: Silica-laden phagocytes in air sacs. *Organization*: Nil. *Distribution*: Diffuse. *Fibrosis*: Nil. *Comment*: This experiment illustrates the preventive effect of shale. When mixed with quartz, the latter produces practically no fibrosis but remains unorganized, lodged in loose koniophages. This picture is more or less uniform throughout the lungs of all animals in the experiment. Shale apparently depresses the fibrogenic effect of quartz.

(Reproduced from "Chronic Pulmonary Disease in South Wales Coalminers, I.—Medical Studies." *Spec. Rep. Ser. Med. Res. Coun., Lond., No. 243, 1942.*)



FIG. 32.—*Dust-reticulation* (dust reticulosis, see p. 42), large section of lung unstained ( $\times 1.5$ ), from case P.135 (anthracite collier for 41 years), showing cobweb-like black dust deposits, also emphysema. There is some oedema present, which makes lung look partially consolidated, but no nodules and no silicotic fibrosis. Total silica, 1.19 per cent. dry lung; ratio of coal to silica about 2 : 1.

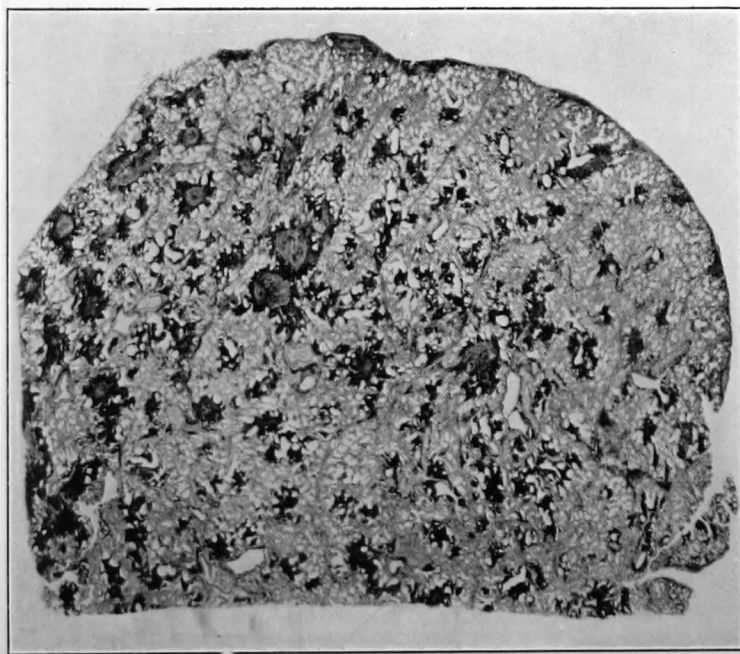


FIG. 33.—Large section of lung ( $\times 1.5$ ) from case P.147 (anthracite collier for 32 years), showing (1) *dust-reticulation* (dust reticulosis, see p. 42), (2) a few *mixed nodules*, some with "medusa-head" effect. The nodules are sporadic in distribution.

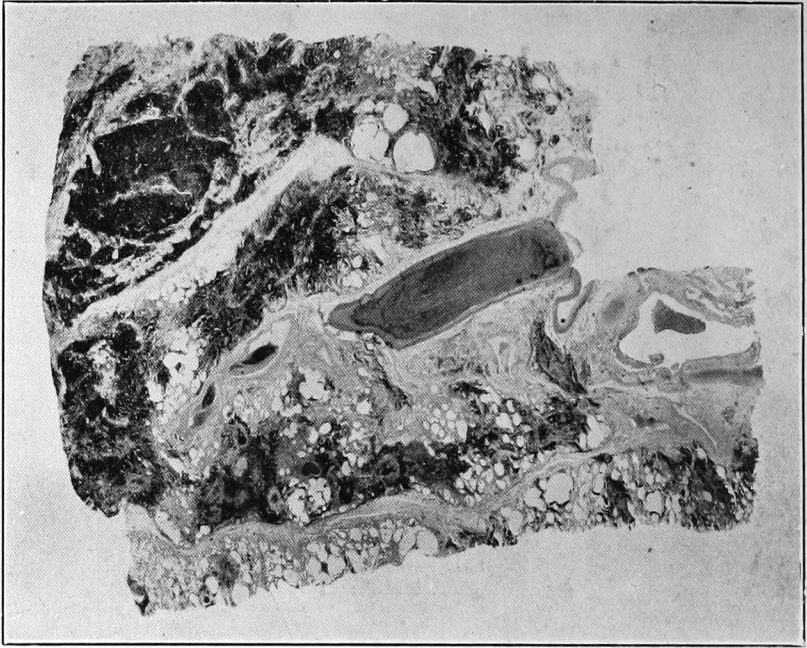


FIG. 34.—Large section of lung ( $\times 2$ ) from case P.117 (screens worker for 21 years), showing *confluent black fibrosis* composed chiefly of dust-reticulation (dust-reticulinosi, see p. 42) but including a few streaks and nodules of silicotic type. Large clot (embolus) in branch of pulmonary artery. Total silica, 1.30 per cent. dry lung.

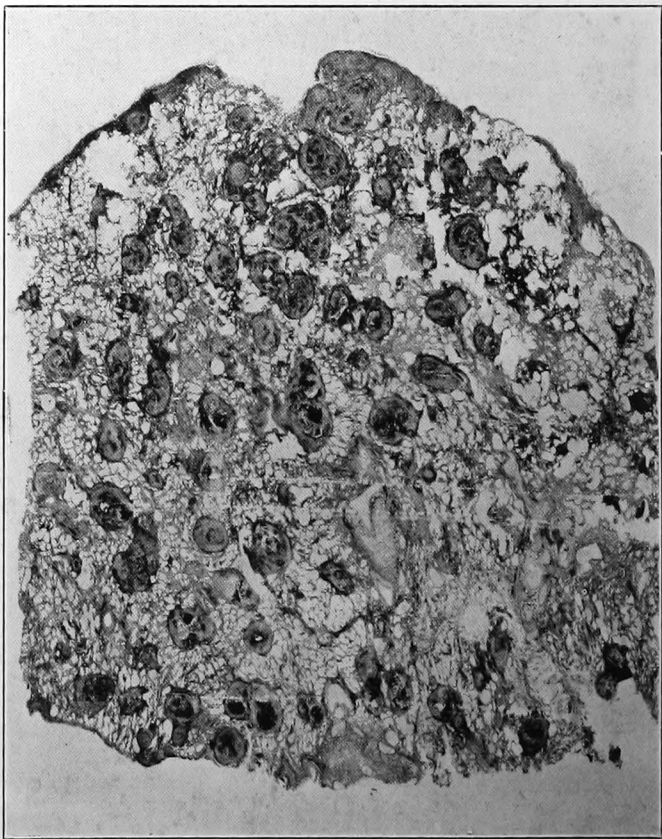


FIG. 35.—Large section of lung ( $\times 1.5$ ) from case P.166 (rock worker for 20 years), showing classical *silicotic nodulation*, not much dust-reticulation, (dust-reticulinosi, see p. 42), marked emphysema. Many of the nodules have granular, necrotic centres. Total silica, 1.06 per cent. dry lung. Ratio of coal to silica dust = about 1:4.

# CHRONIC PULMONARY DISEASE IN SOUTH WALES COALMINERS.—III. EXPERIMENTAL STUDIES

## E.—THE SOLUBILITY OF DUSTS FROM SOUTH WALES COALMINES

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University of London*)

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### INTRODUCTION

In 1923 Gardner published the results of his classic experiment with the finely divided powder of carborundum, a compound of silicon, which failed to produce silicosis despite the sharp-edged nature of its particles. Since Peacock's (1860) and Greenhow's (1865) description of "sand" in the lungs, the injury to the tissues in silicosis, and the consequent fibrous scar-tissue which developed, had been attributed to the sharpness of the grains of sand and to their abrasive action on the soft alveolar and lymphoid tissue of the lung. The merit of Gardner's discovery lay in the failure of a highly abrasive dust, of extreme hardness, to produce the expected well-known fibrous condition of the lungs which had come to be associated in the minds of clinicians and pathologists with the inhalation of siliceous dust.

It was Kettle's (1932) work, however, which appeared to have finally discredited this mechanical theory of silicosis. The powdered minerals quartz and flint had been used by many experimentalists to induce silicotic lesions in the tissues of animals. Kettle himself had been a notable contributor in this field. He found, however, that the coating of a previously harmful quartz dust with an impervious layer of iron oxide rendered it quite innocuous when introduced

into the lungs of guinea-pigs. Since the particles of this iron-coated quartz still displayed their sharp edges under the microscope, and appeared to be as dangerous weapons for wounding the delicate structures of the lungs as they had been before receiving their coating, it was concluded that their great potency in causing pathological change in their uncoated condition could not be due simply to mechanical causes. Gye and Kettle (1922) and Gye and Purdy (1924) had carried out experiments with a soluble compound of silicon, namely silicic acid, and had advanced convincing evidence to show that it was a general tissue poison, and, perhaps more impressively, that the lesions of acute inflammation and necrosis which resulted from its introduction in various sites of the body provided an extremely favourable bed for the proliferation of tubercle bacilli. When it is remembered that the most serious complication of silicosis is tuberculosis, the importance of this discovery will be recognized.

From these experiments with soluble silica, there sprang the idea that silicosis must be due to some toxic action rather than to a trauma of the tissues. The negative results of Gardner with carborundum and of Kettle with iron-coated silica gave the final confirmation. The chemical substance which constitutes quartz is silica, or silicon dioxide. Its chemical formula is  $\text{SiO}_2$ . That of silicic acid is  $\text{H}_2\text{SiO}_3$ ,\* and it will be readily appreciated that the acid is, like many other mineral acids, simply the hydrated form of the oxide of the element; or—to put it in converse terms—that silica is the anhydride of silicic acid, i.e.,  $\text{SiO}_2$  results from the subtraction of  $\text{H}_2\text{O}$  from  $\text{H}_2\text{SiO}_3$ . The hydration of silica in the tissues, therefore, is the *sine qua non* of this “toxicity theory” or “chemical theory” of silicosis; silica must become hydrated and form silicic acid before it can do harm. It must be rendered soluble to produce its characteristic fibrous response; hence the “solubility theory” of silicosis. These three expressions mean the same thing, i.e., that a soluble chemical substance—silicic acid, formed by hydration of the surface of the quartz or other siliceous particles, is the real causative agent in silicosis.

The natural corollary of this theory is that the more soluble a siliceous dust is, the more toxic it might be expected to be. Generally speaking, this appears to be the case. Quartz, flint, chalcedony, opal, are much more soluble, and much more silicosis-producing, than kaolin, mica, talc and fuller's earth. (Briscoe, Mathews, Holt and Sanderson, 1937; King and McGeorge, 1938.) Moreover, the natural mixtures of quartz with kaolin and mica which constitute the shales which have been used in a powdered form for rock-dusting in coal mines have apparently but little power to produce silicosis in miners (Haldane, 1917), and their solubility in body fluids is low (Whitehouse, 1937; King and McGeorge, 1938).

The quartz contained in these natural shale dusts is often present in sufficient amount to yield a much higher concentration of dissolved silicic acid than is actually found (Whitehouse, 1938), and their admixture with quartz or flint causes an apparent depression of the solubility of the latter in artificial and body fluids (King and Roman, 1938).

Considerations of a more theoretical nature had led Denny, Robson and Irwin (1937) to seek a depression of quartz solubility by metallic aluminium. This they found, in fact, to result from the inclusion of almost incredibly small amounts of finely powdered aluminium metal, or of larger amounts of the oxides and hydroxides of aluminium. The nature of the depressant action was shown to be a coating of the quartz particle with a crystalline oxide of aluminium. This layer presumably protected the siliceous particle from any action by the liquid bathing it, as did the iron oxide in the case of Kettle's experiment, and

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\* The use of meta-silicic acid for explanatory purposes is a simplification. There are other silicic acids as well. The matter is more fully discussed elsewhere (King and Belt, 1938).

it had the same—or, indeed, a much more pronounced—protective action when the alumina-coated quartz powder was inoculated into animals. The inclusion, moreover, of aluminium dust in the powdered quartz breathed by experimental rabbits, appeared to prevent the development of silicosis in their lungs. This prevention of silicosis has been taken by many to mark the final proof of the "solubility theory": The mechanism of the prevention furthermore suggested an explanation of the long-known protective action of the powdered shales used for stone dusting, and Bremner (1939) showed that the same release of alumina from the protective dusts took place as from metallic aluminium, and was followed by the same coating of the quartz particles, thus vindicating Haldane's theory of "antidotal rocks".

But successful as the solubility theory may be in explaining the observed facts of silicosis and in indicating new lines of approach, it must be recognized that it does not always appear to work out in practice. While, in the main, it is true that the more soluble forms of silica are the more toxic, it is not true that the most soluble is the most toxic. Gardner (1938) found that the extremely soluble "20 Angstrom silica", produced as a condensate in furnaces where silica sand is melted, is without effect on the lungs and livers of experimental animals. And it is a matter of long industrial experience that workers in factories using such furnaces do not appear to suffer unduly from affections of the lungs.\*

At the other end of the solubility scale are dusts which are anything but non-toxic, despite their low *in vitro* silica solubilities. Many of the coal measure sandstones, (e.g., Somerset greys and the Pennant rock) have long been thought, on good industrial experience, to form dangerous dust when drilled and otherwise worked, and this despite the fact of their belonging to low silica-solubility species (cf. King, McGeorge and Roman, 1938). In the present report, moreover, there are descriptions of animal experiments with dusts of low solubility (e.g., stratum A of mine A3) which indicate a pathogenicity almost comparable with that of the very much more soluble Brazilian rock-pebble, the form of quartz used in this investigation as a sort of base-line for both animal and solubility experiments.

Another discrepancy with the solubility theory is seen in our failure to find any marked difference in the solubilities of South Wales dusts of known difference in pathogenicity. The South Wales shales and sandstones from the coal mines contain variable amounts of quartz, mica and kaolin. Despite wide differences in the proportions of these minerals, their solubilities are all very low and very similar; and there is little difference between the silica solubilities of the powdered rocks and air-borne dusts from the anthracite mines which have a high incidence of silicosis, and from the steam coal and bituminous mines which have a low incidence. We have, in fact, failed to find the difference in silica-solubility between the dusts and powdered strata from the anthracite and bituminous fields which their known differences in pathogenicity for the miner might have led us to expect, and we are driven to conclude that there must be some difference in the nature of the dusts which has thus far proved too subtle for our test-tube experiments to detect. Further comment on these differences, and on the failure of chemical or animal experiments to resolve them, will be found in the Discussion (p. 89). For the moment, it is sufficient to remark that the solubility theory explains many, but not all, of the facts of silicosis; and that when applied to the more complex form of pneumokoniosis found in South Wales (Hart and Aslett, 1942; Belt and Ferris, 1942), it fails to account for the great variability in the severity of lung disease found in the mines. When

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\* Bruce (1937) has reported from Sweden that in the manufacture of silicon alloys some cases of silicosis have developed owing to the inhalation of condensed particles of silica in a particularly dusty atmosphere.

applied to the known constituents of mine dust, it suggests that those dusts with most quartz in them should be most pathogenic and those with more mica and kaolin, or coal, should be less so; and in the main this seems to be the case. But the solubilities of the mixtures of these minerals which are found in the mine strata and dusts are not grossly different as would be expected; they are surprisingly similar. While we are confident that some chemical dissimilarity must exist, we have not yet been able to identify it.

The results presented are selected from a large mass of experimental data which have accumulated over the last six years. They represent part of our attempts to find a chemical explanation, in terms of silica solubility, of the South Wales pneumokoniosis; while they have failed in some respects, they have yielded useful information in others, and facts of scientific interest have emerged which may be of use in explaining previously puzzling findings, particularly in experimental silicosis.

### EXPERIMENTAL METHODS

*Preparation of Dusts.*—The dusts used in these experiments were of two types. Those which were artificially prepared from samples of rock received from the mines, and the collected samples of air-borne dusts produced by drilling, shot-firing, etc., in the mines.

The production of a dust from gross specimens of the stone and coal strata proved to be much more difficult than had been anticipated. Grinding of selected pieces of rock in a steel machine resulted in the contamination of the dust with a considerable proportion of finely divided steel. As iron has a depressing effect on the solubilities of silica-containing dusts (King and Roman, 1938), these specimens could not be used for solubility or animal investigations.

Pulverization in the special apparatus used by the Micrometric Reduction Company, in which the lumps of rock are propelled around an annular chamber by jets of air and produce a dust by impinging on one another, resulted in powders of very fine and uniform particle size. In the case of pure mineral specimens, the powder was admirably suited to our purpose, but with shale and sandstones the softer components of the rock appeared to be eroded from the lumps more readily than the harder components, and to be concentrated in the dust carried over in the air stream.

After many trials with this and other types of apparatus, we finally succeeded in producing a dust of the requisite fineness, and whose composition appeared to be invariably identical with that of the parent rock specimens, by slowly rotating walnut-sized pieces of rock in a rubber-lined steel "tumble-mill". This was box-shaped, 1 foot long by 8 inches square, and was lined with rubber (the lining was kindly carried out for us by Mr. Johnson of the British Tyre and Rubber Co.). One side of the box was detachable and held in place by bolts. By introducing washers, this side was arranged so as to leave a small space between it and the body of the box, through which the dust could fall as it was produced. The whole was mounted in a tight wooden container and made to rotate at about 50 r.p.m. Under these conditions, considerable quantities of very finely powdered rock could be produced in two to three days. All the specimens appeared to be entirely free from contamination by rubber,\* and during several years of almost continuous use the mill has appeared to show no evidence of erosion of its rubber lining. The mill is shown in Fig. 36. In all this work we were greatly assisted by Dr. A. Brammall, who examined the dusts produced for their mineralogical and chemical composition.

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\* It is absolutely essential that the mill be rubber-lined. A steel mill releases particles of metal into the dust, and this results in a lowering of the apparent silica solubility figures. Thus, two samples of quartz powder, prepared in a tumble-mill before and after it was lined with rubber, gave 7.3 mg. SiO<sub>2</sub> in solution per 100 ml. for the sample from the rubber-lined mill and only 2.4 mg. from the unlined mill.



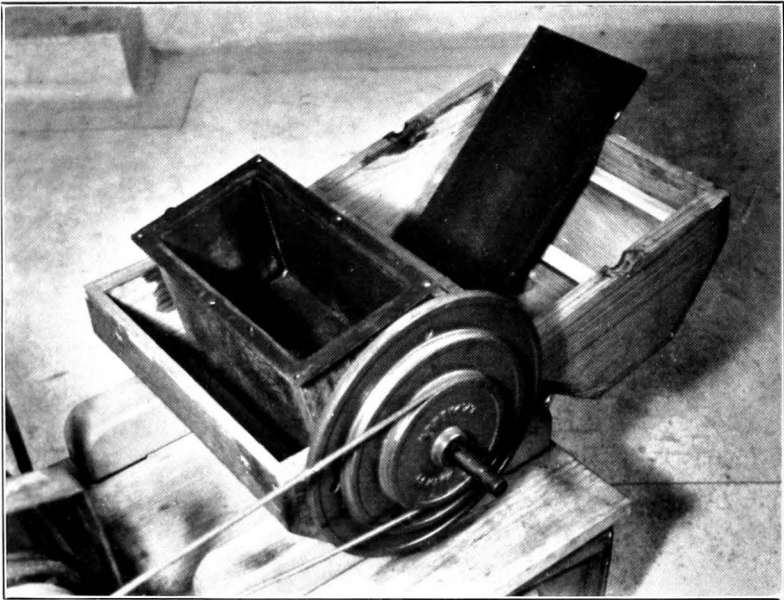


FIG. 36. Rubber-lined tumble-mill for powdering minerals (see p. 72).

The air-borne dusts were collected in the mines in the various places of working by Professor H. V. A. Briscoe and his colleagues, and by Mr. D. Hicks. The methods of collection used, and the composition of the specimens, have been fully described in other papers (*M.R.C. Special Report Series No. 244*). All specimens of dusts and powders used for the solubility studies were examined by Drs. T. Bedford and C. G. Warner for their particle sizes, and the results obtained are given in full on Table XXXVI, p. 88, and in an abbreviated form in the accompanying communication by Belt and King.

*Ageing of Dusts.*—The question whether a dust should be used for animal experiments and for solubility studies directly it has been formed or at some later time caused us some initial concern. Denny, Robson and Irwin (1937) had claimed to show a much more ready production of experimental silicosis by exposing animals to freshly formed quartz dust than they had previously obtained with "stale" dust; and Briscoe, Mathews, Holt and Sanderson (1937) adduced evidence to show that freshly ground minerals yielded more silica in solution in boiling water than the same specimens after they had been aged by preservation in bottles for some time.

The dust breathed by miners is, of course, partly fresh and partly old. Operations such as shot-firing, drilling, shovelling, etc., disturb the deposits of dust from floor, walls, rafters, etc., as well as producing new dust. These deposits are constantly being added to, and are constantly being disturbed. The air-borne dust, therefore, contains only a proportion of freshly formed particles. Nevertheless, the possibility of freshly fractured surfaces having something about them, e.g., in the way of unsatisfied valencies which might cause the release of more silicic acid to the tissues than could result from particles which had been exposed to air and moisture, has to be considered in the investigation of pneumokoniosis, both in the test-tube and in the animal.

Dusting experiments with animals, in which guinea-pigs were exposed to dust freshly produced from pieces of sandstone and of a shale stratum, by means of the tumble-mill shown in Fig. 36, were quite unsuccessful, and this form of experiment was finally abandoned for this and other reasons which are discussed in the section by Belt and King above.

Solubility experiments with freshly prepared samples of powder (ground in an agate mortar and in a porcelain ball-mill) gave equally negative results. Thus, freshly ground sandstone (stratum A of A3), the particles of which were not more than an hour old before being added to the liquid, released 1.0 mg. of silicic acid into 100 ml. of Ringer's solution (pH 7.4) when an excess of the powder was shaken at 37° with the liquid for 48 hours. Dust from the same pieces of rock, which had been prepared six years previously and preserved at room temperature, released 1.2 mg. under the same conditions. The dust from a shale stratum (C of A3) similarly gave 1.4 mg. for a fresh specimen and 1.3 mg. for a six-year old one. It appears, therefore, for the few specimens of South Wales rock which we have so tested, that there is no evidence for either a greater solubility or a greater pathogenicity in the freshly-formed dusts. We recognize, however, that our experiments give at best a very artificial imitation of the mutual influences, one on the other, of dust, fluid and tissue in the lung, and that what may fail to produce a positive result *in vitro*, or in an animal within a few months, may well do so in a human lung over a long period of years.

*Liquids for Solubility Studies.*—The ideal liquid for studying the solubility of siliceous dusts is blood plasma, but it is difficult to use, because of its viscosity, which tends to increase on long time shaking at 37°. Its free phosphate and high protein content necessitate extra preliminary treatment before the dissolved silica can be determined; and bacterial contamination is difficult to control. King and McGeorge (1938) found that ascitic fluid gave almost the same results with various siliceous dusts as did blood plasma (see also Table XXI).

TABLE XXI

*Dissolution of Silica from Mixtures of Quartz and Mineral Dusts in Blood Serum and Ascitic Fluid*

(0.2 g. of dust shaken with 10 ml. liquid for one week at 37° C.)

	mg. SiO <sub>2</sub> dissolved per 100 ml.		
	Blood serum.	Ascitic fluid.	Deproteinized ascitic fluid.
Quartz .. .. .	7.4	7.4	7.8
Kaolin .. .. .	0.9	0.7	1.2
Kaolin + quartz (1 : 1) .. .. .	3.6	3.7	3.8
Chlorite .. .. .	1.7	1.5	1.4
Chlorite + quartz (1 : 1) .. .. .	2.7	2.4	1.6
Stone dust .. .. .	0.8	1.1	1.1
Stone dust + quartz (1 : 1) .. .. .	1.9	2.0	1.9
Air-borne mine dust D .. .. .	1.3	1.4	—
E .. .. .	2.0	1.1	—
I .. .. .	1.3	1.8	—
J .. .. .	1.2	1.4	—
Quartz + alumina (50 : 1) .. .. .	0.5	0.3	0.4

Its electrolyte content is almost identical with that of serum, and because of its very much lower protein content, it is much easier to handle in the analytical procedure. The deproteinizing and dephosphating steps are not, however, eliminated with ascitic fluid, and some protein-free buffered solution which does not contain inorganic phosphate has been sought as a more convenient medium for estimating silica solubilities. Unfortunately, there are relatively few buffers covering the physiological pH range which do not contain phosphate. Many have been tried, and a few have been found to give results similar to those with blood serum and ascitic fluid. Others have been discarded for various reasons, and particularly because of interference by the buffering substance with the reduced-molybdate method for the colorimetric estimation of dissolved silica. In Table XXII are given some comparative results for several of

TABLE XXII

*Effect of Different Buffered Solutions on the Dissolution of Silica from Quartz and Shales*

(0.2 g. of powder shaken with 10 ml. of liquid for 48 hours at 37° C.)

	mg. SiO <sub>2</sub> dissolved per 100 ml.				
	Quartz.	A3 shale.	A1 shale.	Quartz + anthracite coal.	Quartz + steam coal.
Ringer's solution pH 7.4 .. .. .	6.3	1.3	1.1	—	—
1 per cent. NaHCO <sub>3</sub> adjusted to pH 7.5. .. .. .	6.3	1.3	1.4	—	—
Glyoxaline buffer pH 7.2 .. .. .	6.2	1.6	1.2	—	—
Veronal pH 7.4 .. .. .	6.4	1.4	0.8	2.0	5.5
Ascitic fluid pH 7.6 .. .. .	6.0	1.3	1.3	2.1	5.2
Ultrafiltered ascitic fluid .. .. .	6.2	1.4	1.3	—	—

the fluids and buffers which have been used. It will be seen that with a variety of mineral dusts the results are in substantial agreement, and it seems permissible to compare solubility figures for one of these liquids with those for any of the others. The Ringer solution described is the best liquid on theoretical grounds, since it most closely approximates to an ultra-filtrate of blood plasma, but it is a poor buffer and, for this reason, we have found it necessary to include with it some other substance of higher buffering capacity. For this purpose, the glyoxaline buffer of Kirby and Neuberger (1938) is recommended as being the most satisfactory.

A modified Ringer's solution, from which phosphate had been omitted, was prepared as follows: 10 g.  $\text{NaHCO}_3$ , 0.42 g.  $\text{KCl}$ , 0.48 g.  $\text{CaCl}_2$ , and 2.0 g. of glucose (all of "Analar" quality) were each dissolved in water. The solutions were mixed, diluted to nearly 1 l., and adjusted to pH 7.4 by dropwise addition of hydrochloric acid. The mixture was diluted to 1 l. and filtered from the small precipitate of calcium carbonate which always formed on mixing the solutions of  $\text{NaHCO}_3$  and  $\text{CaCl}_2$ .

The "universal buffer" mixture of Thiel, Schutz and Coch (1934) was used for certain experiments in which a wide range of pH was investigated in relation to silica solubilities of several minerals. This mixture appears to be the only buffer so far described which has a wide pH range and contains no phosphate. By mixing the following solutions in the requisite proportions, buffers are obtained with pH's ranging from 1.5 to 11.0:—

- (1) 0.05 molar oxalic acid and 0.02 molar boric acid.
- (2) 0.02 molar boric acid, 0.05 molar tartaric acid and 0.05 molar sodium sulphate.
- (3) 0.05 molar borax.
- (4) 0.05 molar sodium carbonate.

The presence of the borate and tartrate has a depressant effect on the blue colour in the King (1939) silica method, but by allowing an hour instead of the usual 15 minutes for the colour development, this interference was reduced to a minimum.

Veronal (Michaelis, 1930) likewise retards the development of colour in the silica method, and experiments with this buffer were adjusted accordingly.

*Mixture of Mineral Dusts with Liquids for Solubility Studies.*—The proportion of powdered mineral to be used in a given amount of solvent in order to produce the maximum solubility figure was studied by King and McGeorge (1938), who found that with quartz dust, the maximum silica solubility figure was reached with 1 per cent. suspensions in about two days. With 0.5 per cent. suspensions of quartz, equilibrium was attained more slowly, but the same final figure for apparent solubility was reached. With larger concentrations of dust, the maximum solubility was reached more quickly. But increases over 1 per cent. do not alter the final figure for mg.  $\text{SiO}_2$  dissolved. They only hasten its attainment.

This is not to say, of course, that, with mixed dusts containing only a proportion of soluble silicate mineral, suspensions of more than 1 per cent. of the dust in the solvent will not yield a higher silica solubility figure. They obviously will, if a large part of the dust is inert and only a small part is soluble. The choice must be made, therefore, between using a constant proportion of dust to liquid, and of using such an amount of dust as will always yield the same proportion of the constituent or constituents which liberate  $\text{SiO}_2$  in the solution. The latter alternative is impossible, for the reason that one cannot know what will be the solubility behaviour of all the constituents of a given dust, or the effect of one constituent on the behaviour of another. Furthermore, it is obviously a better imitation of conditions in the lung to maintain a constant proportion of solid to liquid rather than to vary the former. So far as is known,

the lung does not show much discrimination between different mine dusts, and the mixture of a large amount of an inert diluent with the siliceous matter should mean that there is less of the siliceous component present in the lung, and that the total concentration of dust is much the same. In the lung, therefore, the ratio of mineral matter to the liquid which bathes it will be largely independent of the percentage of quartz or other siliceous component in the dust present.

In all our previous work, we have used mineral dust in the proportion of 2 g. per 100 ml. of solvent. This condition produced the maximum result with quartz and other forms of free silica in two days, and gave the maximum figure for other silicates studied in the same time. A like condition has been adhered to in all the determinations described in this report. The usual experiment involves the use of duplicate samples of 0.2 g. of the dust in 10 ml. of solvent in non-glass test tubes (the "Lusteroid" tubes supplied by Messrs. Gallenkamps have been very satisfactory). These are stoppered with rubber bungs, and are rotated in the 37° C. room for the specified period of time.

The time for shaking the mixture of siliceous dust and solvent is ideally about a week (King, McGeorge and Roman, 1938), but in many of our experiments we have used a shorter time, in order to expedite the determinations. A period of 48 hours is ample to give comparative figures for different dusts, and this has been used for most of the work here described.

*Estimation of Dissolved Silica.*—The molybdate blue colour method of King (1939) has been followed throughout. Deproteinization, and the elimination of phosphate, were accomplished by the basic ferric acetate method. With solvents which did not contain protein or phosphate, this step was unnecessary. It was necessary, however, to neutralize the samples for colorimetric analysis. This was done with *N* sulphuric acid and methyl orange as indicator.

The separation of the excess dust from the solvent was accomplished, in previous investigations, in an ultracentrifuge. In the present study, this step has been omitted and an hour's centrifuging in a Swedish angle centrifuge has been substituted. The centrifuged sample was poured through a No. 44 Whatman filter paper, and if the filtrate did not appear crystal clear, it was re-centrifuged and re-filtered.

All results have been expressed in terms of silicon dioxide ( $\text{SiO}_2$ ) dissolved per 100 ml. of the solvent used.

## RESULTS

*The Effect of pH on the Solubility of Mine Dusts.*—The various constituents of mine dusts should affect silica solubility in different ways at different acidities. Free silica, being composed of the weak acid, silicic acid, will tend to be more strongly attacked by alkaline fluids than by acid ones; the silicates will be attacked by strong acids (and bases), and should be fairly stable at reactions approximating to neutrality; an amphoteric substance like alumina will exhibit its minimum solubility near neutrality, and should there exert its maximum depressant effect on the amount of silica dissolved; at more acid and alkaline pH's, alumina will tend to dissolve, either as a salt or as an aluminate, and its interference with the dissolution of silica should tend to disappear; free iron, on the other hand, should depress silica dissolution at all non-acid pH's, since it does not tend to dissolve in alkali.

On this reasoning, a free silica such as quartz should be less soluble at acid pH's, more soluble at alkaline. Kaolin should exhibit its minimum solubility near neutrality; so, likewise, should a mixed mineral like shale. Such results are illustrated in Table XXIII.

The more complex situation in which aluminium is released from a mixed dust to coat the surface of quartz (see below) is shown in Table XXIV.

TABLE XXIII

*Effect of pH on Silica Solubility**(0.2 g. of powdered mineral shaken with 10 ml. of buffer for 18 hours at 37° C.)*

pH.	mg. SiO <sub>2</sub> per 100 ml.		
	Quartz.	Kaolin.	A3 shale.
3	0	1.0	—
4	0.07	0.8	2.5
5	0.09	0.6	2.3
6	0.27	0.6	1.8
7	2.4	1.1	1.1
8	2.9	1.2	0.8
9	4.6	1.7	0.9
10	5.0	3.2	1.4
11	5.4	—	—

Whereas quartz tends to be more soluble at pH 7 than at pH 2 or 3, the figures for the mixtures of quartz with alumina or the shales show a minimum of silica dissolved at or near to pH 7. This is due to the removal of its protective action at more acid pH's, owing to its dissolving in the acid buffers to form aluminium salts. Likewise, at pH's above 11 there is a tendency to form sodium aluminate, thereby removing the alumina coating from the quartz particles.

TABLE XXIV

*Effect of pH on the Dissolution of Silica from Mixtures of Quartz with Aluminium—containing Mineral Dusts**(0.2 g. of mixed dust shaken with 10 ml. buffer for 18 hours at 37° C.)*

pH.	mg. SiO <sub>2</sub> per 100 ml. buffer.					
	Quartz alone.	Quartz + aluminium.	Quartz + alumina.	Quartz + A 3 shale.	Quartz + S 4 shale.	Quartz + B 1 shale.
2	—	—	0.26	—	0.65	—
3	0.22	0.42	0.20	0.41	0.38	1.06
4	0.23	0.27	0.20	0.34	0.29	0.78
5	0.23	0.13	0.20	0.34	0.29	0.70
6	0.38	0.11	0.23	0.34	0.30	0.70
7	1.5	0.10	0.27	0.56	0.54	0.65
8.5	5.9	0.10	0.33	1.0	1.6	0.83
10	7.1	0.15	0.39	1.2	4.2	1.1
11	—	0.17	1.2	4.5	6.6	2.3
12	7.4	0.18	1.4	5.4	7.0	2.9
13	10.0	0.86	7.7	8.6	12.2	10.0
14	—	5.4	—	—	—	—

Such results, however, probably have little bearing on the fate of mineral dusts in the lungs, where the pH of the fluid bathing the particles cannot be far removed from that of the plasma of venous blood, viz. pH 7.2. There is a widespread misapprehension that, because carbon dioxide is given off in the lungs, the fluid there must consequently be saturated with CO<sub>2</sub> and thus have an acid reaction. But the CO<sub>2</sub> tension in the alveoli is only about 40 mm. of mercury, and the dry alveolar air contains only about 5½ per cent. of carbon dioxide, so that, far from there being a high concentration of CO<sub>2</sub> in the lungs, there is really only a very small one.

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In the stomach, the hydrochloric acid of the gastric juice exerts an action on silicates and on mixed dusts, and the alkaline intestinal juice dissolves the silicic acid so formed (Whitehouse, 1938). In this way, silicates and mixed shale dusts introduced into the alimentary tract may yield more silicic acid into the blood stream than they would if shaken with blood plasma or when they are present in the lungs, but silicic acid absorbed from the gut appears to be promptly excreted by the kidneys (King, Stantial and Dolan, 1934), and probably produces no injurious effect in the body.

The experiments summarized in Tables XXIII and XXIV were for shorter times than were generally used, and the silica solubility figures at pH 7 are consequently much smaller than those shown for other experiments, where the time allowed for dissolution was longer.

*Effect of the Mineral Content of Coalmine Dusts.*—On first thought, it would appear that the more siliceous mineral matter there is in a mine dust, the more silica it should yield in solution. From what has been said, however, it will be clear that this is not necessarily the case. Certain silicates yield aluminium in solution, and this depresses the solubility of other siliceous components, particularly free silica, in a dust. Moreover, some of the South Wales dusts may contain free alumina and possibly free iron (cf. *M.R.C. Special Report Series*, No. 244, 1943, p. 144), and these will markedly retard the passage of silicic acid into solution. On the other hand, small amounts of lime may enhance the dissolution.

The lack of any correlation between the silica solubilities of four mine dusts and their ash contents (most of the ash is siliceous) or their silica content, is shown in the following figures: dust D (16 per cent. ash, 49 per cent.  $\text{SiO}_2$ ) yielded 1.4 mg.  $\text{SiO}_2$  in 100 ml. of ascitic fluid, dust I (18 per cent. ash, 51 per cent.  $\text{SiO}_2$ ) yielded 1.8 mg.  $\text{SiO}_2$ , dust J (25 per cent. ash, 51 per cent.  $\text{SiO}_2$ ) yielded 1.4 mg. and dust E (39 per cent. ash, 54 per cent.  $\text{SiO}_2$ ) 1.1 mg. The amount of ash in a mine dust may, however, have a very pronounced effect on the extent to which it will depress the solubility of quartz, as will be shown later.

*Comparative Silica Solubility Figures for all South Wales Dusts Investigated.*—For the reasons discussed under "Methods", a standard percentage of dust in the liquid used as solvent was employed for the comparative solubility studies. All the dusts used for the animal experiments, and various others which were collected or prepared from time to time, have been tested for their silica solubilities at several times and in several solvents. No significant variations from a low average solubility of about 1 mg.  $\text{SiO}_2$  per 100 ml. have been found among the dusts from different mines, or among dusts of great difference in composition from the same mine, or in the same dusts tested on different occasions. All have yielded a minimum of silicic acid into solution, and this despite the known incidence of pneumokoniosis resulting from their inhalation, the fact that several of them are of proven pathogenicity in animal experiments, and that at least one of them (A of A3) has a pathogenicity almost equal to that of quartz. Results obtained with all the dusts in two solvents are given in Table XXV.

When the quantity of dust is adjusted so as to contain identical amounts of silica, the figures for dissolved  $\text{SiO}_2$  are still low, but are somewhat more uniform. Some results with air-borne dusts and the dirt (extraneous mineral matter) from the coal seams are given in Table XXVI. It will be noted that the use of more dust per unit of solvent has slightly increased the figures for mg. of  $\text{SiO}_2$  dissolved, and that there appears to be a somewhat orderly ratio between solubility and grade of coal, the anthracite dusts being less soluble and the bituminous more so. This, it should be remarked, is the reverse of what would be anticipated on the solubility theory of pathogenicity, but is the same

sort of relationship which will be seen in the extent to which the different coals depress quartz solubility.

TABLE XXV

*Dissolution of Silica from a Standard Amount of all Dusts tested.  
(0.2 g. of dust shaken with 10 ml. liquid for 48 hours at 37° C.)*

	mg. SiO <sub>2</sub> dissolved per 100 ml.		
	Ascitic fluid.	Ringer's solution buffered at pH 7.2 with glyoxaline.	
<i>Strata of A3 mine—</i>			
A, sandstone .. .. .	0.9	1.2	1.3
B, clift (shale) .. .. .	1.0	1.6	1.8
C, clod (shale) .. .. .	1.2	1.6	1.8
D, upper bord coal .. .. .	1.1	0.8	0.9
E, lower bord coal .. .. .	1.3	0.4	0.5
F, clod (shale) .. .. .	1.4	1.9	1.9
G, coal (working seam) .. .. .	1.3	0.3	0.4
H, fireclay .. .. .	1.1	—	—
<i>Clod (shale) from other mines—</i>			
A1—94LPJ .. .. .	1.2	1.2	1.1
A1—94LPF2 .. .. .	1.1	1.3	1.1
S4 .. .. .	0.9	0.8	0.8
<i>Extraneous mineral matter from coal seams—</i>			
A3 (34 per cent. ash) .. .. .	—	0.3	0.2
A1 (33 per cent. ash) .. .. .	—	0.3	0.3
A2 (32 per cent. ash) .. .. .	—	0.3	0.4
S4 (47 per cent. ash) .. .. .	—	1.4	1.4
<i>Air-borne dusts—</i>			
A3—D .. .. .	0.8	0.7	—
A3—E .. .. .	0.7	0.6	0.4
A3—I .. .. .	0.8	—	—
A3—J .. .. .	0.6	—	—
A3—2HI4 .. .. .	0.2	0.3	0.3
A1—2M1 .. .. .	0.3	0.3	0.3
S4—1U1 .. .. .	0.5	0.6	—
B2—IC1 .. .. .	—	0.3	0.3
B3—1B1 .. .. .	—	0.5	0.6
A3—91US28 .. .. .	0.2	0.1	0.1
A1—94US10 .. .. .	0.6	0.2	0.2
A2—95US10 .. .. .	0.5	0.2	0.2
S4—83US10 .. .. .	0.3	0.1	0.1
<i>Isolated fractions from mine dusts—</i>			
A3—Fine fractions (illite) .. .. .	1.4	1.1	1.3
A1—Fine fractions (illite) .. .. .	1.7	1.4	1.4
S4—Fine fractions (illite) .. .. .	1.3	1.4	1.3
<i>Pure minerals—</i>			
Quartz—Brazil .. .. .	5.9	6.2	—
Quartz—Klondyke .. .. .	6.6	—	—
Quartz—Ontario .. .. .	6.7	6.8	—
Vitreous silica .. .. .	7.6	—	—
Kaolin .. .. .	0.7	0.3	—
Ogofau sericite .. .. .	—	2.9	—
Ohio sericite .. .. .	1.4	1.4	—
Biotite .. .. .	1.7	1.7	—





quartz taken alone. The depressant effect varies from dust to dust and is greatest with alumina. But it is a fact that all the South Wales dusts (as well as many others tested) do depress the apparent solubility of quartz in any fluid of approximately neutral reaction. The extraordinary efficiency with which they do this is illustrated in Table XXVII, where it will be seen that as little as 7 per cent. and as much as 90 per cent. of the silicic acid, which will normally pass into solution, is eliminated by these dusts.

*Depression of Silica Solubility due to Release of Aluminium from the Mine Dusts.*—The great spread in the depression figures points to the existence of some constituent which is present in all of them, but to a variable extent. That there may be free alumina and iron in the shales of South Wales has already been remarked (cf. *M.R.C. Special Report Series*, No. 244, 1943), and that the agent responsible for depressing quartz solubility is alumina seemed probable from analogy with the work of Bremner (1939). All the aluminium contained in a mineral mixture is not, however, available for release into solution, and it seems unlikely that the percentage of total aluminium in a rock dust will bear any direct relationship to the depressant action of that dust on quartz solubility. This point is illustrated in Table XXVIII, where the

TABLE XXVIII

*Effect of Aluminium Content of Mineral Dusts on their Depressant Effect on Dissolution of Quartz*

(0.2 g. of quartz shaken with 0.1 g. of dust in 10 ml. ascitic fluid at 37° C.)

<i>Mineral dust mixed with quartz.</i>	<i>Depression of quartz solubility (per cent.)</i>	
	<i>(1 day.)</i>	<i>(6 days.)</i>
Pennant rock dust (5.9 per cent. Al) .. ..	37	56
Somerset greys dust (7.2 per cent. Al) ... ..	28	32
P. and P. stone dust (9.2 per cent. Al) ... ..	44	50
D. and C. stone dust (10.8 per cent. Al) .. ..	28	27
C. and W. stone dust (13.5 per cent. Al) .. ..	28	32

analyses of several mixed mineral dusts are set out against the extent to which they depress silica-solubility. Figures are given for the extent of the depression at 1 day and at 6 days, and it will be seen that there is not as much difference as might have been expected. Evidently, the depressing agent (aluminium) dissolves from the mineral dust and exerts its effect on quartz fairly promptly, and thereafter the rate of dissolution of the quartz particles is steady and in a fairly constant relationship to the rate of dissolution of untreated quartz particles.

The "releasable" aluminium, then, is probably the constituent which depresses silica solubility. Unfortunately, there is no ready means of estimating the aluminium released from a mineral mixture to coat quartz particles, other than to analyse the quartz itself for aluminium. Several experiments were attempted along these lines, but no consistent figures could be obtained. It was not possible to test the point by mixing quartz and mine dust in a liquid, and allowing time for the supposed fixation of aluminium on the quartz to take place before measuring it there, because the quartz dust could not be separated from the mine dust once they had been mixed together.

Direct measurement of the aluminium passing into solution in ascitic fluid from several test dusts in preliminary experiments failed to show any useful contrast. Some results are given in Table XXIX. Alumina is a far more effective

TABLE XXIX

*Release of Aluminium into Ascitic Fluid*

(0.2 g. of dust shaken with 10 ml. ascitic fluid for 1 week at 37° C., and the dissolved aluminium determined by precipitation with 8-hydroxyquinoline and bromometric titration)

	mg. Al per 100 ml.
Alumina .. .. .	1.12
Biotite .. .. .	1.36
Chlorite .. .. .	0.81
Kaolin .. .. .	0.45
Somerset greys dust .. .. .	0.95
D. and C. stone dust .. .. .	0.72

solubility depressor than biotite, and yet gave less aluminium in solution. Kaolin gave least soluble aluminium, but its value relative to that for alumina gives no measure at all of their relative effectiveness in depressing the solubility of silica. When the mines dusts were placed in water in dialyzing thimbles, and the quartz in water in the containing beakers, variable amounts of aluminium passed through the semi-permeable membranes and became fixed on the quartz dust. But both spectrographic and micro-chemical analyses of the quartz powders so treated failed to reveal any more than the minutest amounts of aluminium, and there was a complete absence of any relationship between the amounts of aluminium determined and the effectiveness of the dusts as depressors of silica solubility.

A rough estimate of the aluminium which is released from a mineral dust to quartz can, however, be obtained by the aurine staining procedure introduced by Denny, Robson and Irwin (1937). Rounded quartz pebbles from the tumble-mill were placed in covered bottles containing a suspension of the stone dust in distilled water. The mixtures were left at room temperature for three months, and the pebbles were then removed. After having been thoroughly scrubbed with a brush under the water tap, and rinsed with distilled water, the pebbles, which were now glistening white, were submitted to the staining process with aurine tricarboxylic acid in acetic acid-ammonium acetate buffer (pH 5.2). The coating of alumina on the pebbles now caused them to adsorb the dye. After the excess had been washed off, some of them appeared of a pale pink or bright red colour, others remained pure white. The intensity of the colour appeared, in fact, to be a measure of the amount of aluminium which had been given off from the dust, and which had become adsorbed on the surface of the quartz. The pebble which had been soaked in the suspension of metallic aluminium dyed a bright red, that treated with alumina was a rosy pink. Some of the silicates released considerable alumina, others—e.g., kaolin—very little. The mixed shale and stone dusts yielded pebbles which were mildly stained, and even those which had been left with the coal dusts were perceptibly pink.

Figures 37 and 38 are coloured reproductions of quartz pebbles treated in this way. Although no figures can be attached to the results so obtained, they provide a visible record and an approximate estimate of the depressing activity of these mineral dusts on free silica solubility. They do not suggest, however, even in a rough sort of way, the extent to which the various dusts may be pathogenic. It had been hoped that they would, and many of the pebbles do seem to fit the general picture. Aluminium and alumina yield the pebbles which stain most strongly, and these two substances have appeared to be retarders of silicosis; kaolin and Ogofau sericite stain scarcely at all, and in

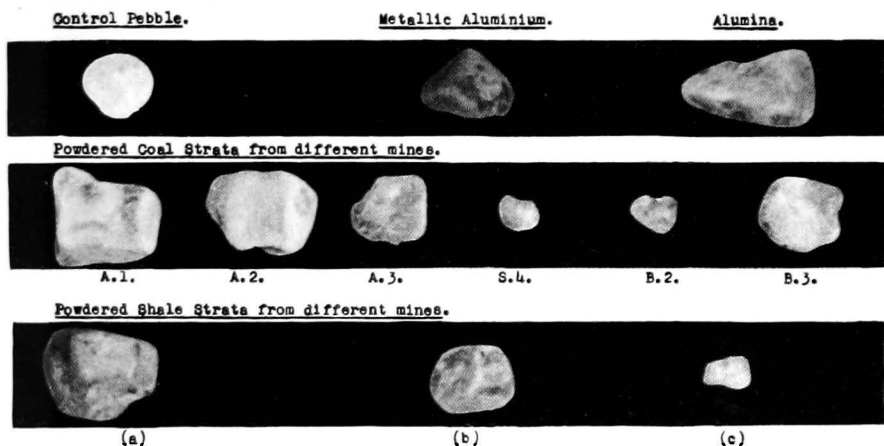


FIG. 37.—Adsorption of aluminium from mineral dusts on quartz pebbles. (Quartz pebbles left in distilled water with mineral dusts for 3 months, removed, well washed and stained with aurine).

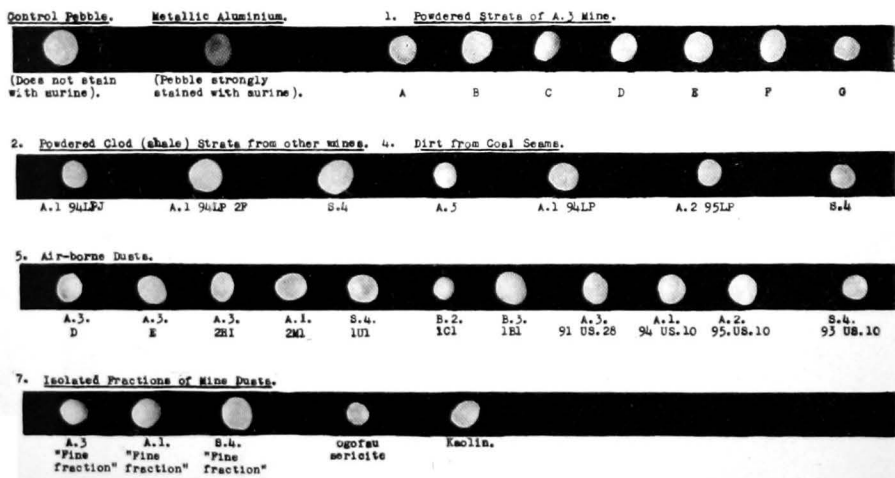


FIG. 38.—Release of soluble aluminium from mineral dusts, as shown by its adsorption on quartz pebbles. (Quartz pebbles left in distilled water with mineral dusts for 3 months, removed, well washed and stained with aurine).

the animal experiments these two minerals produced lesions. On the other hand, the shales and micaceous fine fractions produced pink pebbles and were of minimum pathogenicity in the lungs of rats. But the sandstone stratum A of mine A3 also yielded pink pebbles, and it was one of the most toxic dusts tested. Whether or not a better planned experiment would reveal that it had less total "releasable" aluminium than mildly toxic dusts, can be determined only by further trials and more elaborate investigations.

*Decrease of Dissolved Silica in Presence of Alumina and of Silicates.*—It was shown by King and McGeorge (1938) that, in addition to depressing the solubility of silica, alumina and certain silicates would also remove silica which had already gone into solution. This might be brought about by chemical combination or by an adsorption process analogous to the adsorption of alumina on quartz. If the former, then the amount of silica disappearing from solution should be of a size approximating to that of the alumina or silicate present, and at equilibrium should be in some sort of stoichiometric relationship to the absorbing solid. That the amounts of silica taken up from solution by very finely divided alumina and silicates, well shaken with frequently renewed silicic acid solution, are negligible in comparison with the amount of solid taken, is shown in Figure 39. The silica disappearing from solution was less than 1 mg. in each test, and this is a very small amount to be taken up by 200 mg. of solid. This, together with the shape of the curves, suggests that the phenomenon is one of simple adsorption.

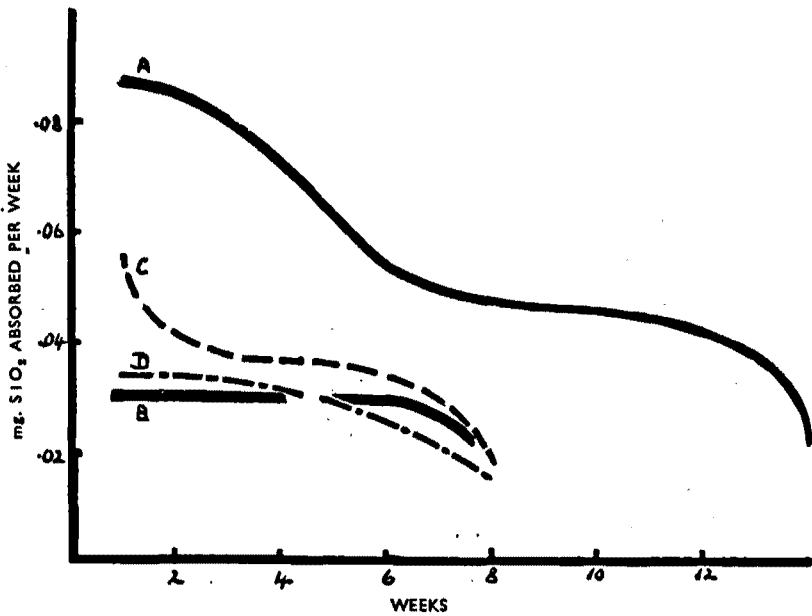


FIG. 39. Uptake of silica by alumina and by silicates. 0.2 g. alumina or silicate dust shaken for 1 week with 1 ml. of silicic acid solution, pH 7.2, containing 0.1 mg.  $\text{SiO}_2$ ; supernatant liquid removed after centrifuging, and the unabsorbed silica estimated; fresh silicic acid solution added and process repeated. Total silica absorbed as follows:

	<i>mg. SiO<sub>2</sub></i>
A. Alumina	0.78
B. Biotite	0.23
C. Chlorite	0.28
D. D. & C. stone dust	0.23

*Depression of Silica Solubility by Coal.*—The interesting idea was suggested to us by Dr. A. K. Schofield and Dr. G. Nagelschmidt that organic matter

containing a considerable number of hydroxyl groups, such as peat or coal, might form a coating over quartz particles, thus depressing their solubility. Experiments devised to test this possibility are shown in Table XXX. The higher the grade of the coal substance, the fewer hydroxyl groups should

TABLE XXX

*Depression of Silica Solubility by Peat and Coal Dusts*

(0.3 g. silica dust, with or without 1.5 g. powdered peat or coal, shaken with 15 ml. liquid for 48 hours at 37° C.; ultra-centrifuged; dissolved SiO<sub>2</sub> determined colorimetrically)

	mg. SiO <sub>2</sub> dissolved per 100 ml.
<i>Ascitic fluid—</i>	
Quartz .. .. .	6.0
Quartz + A1 anthracite .. .. .	2.1
Quartz + S4 steam coal .. .. .	5.2
Quartz + peat .. .. .	5.5
Flint .. .. .	3.4
Flint + anthracite .. .. .	1.6
Flint + steam coal .. .. .	3.4
Flint + peat .. .. .	3.2
Amorphous silica .. .. .	7.9
Amorphous silica + anthracite .. .. .	8.2
Amorphous silica + steam coal .. .. .	8.1
Amorphous silica + peat .. .. .	8.0
<i>Veronal buffer pH 7.4—</i>	
Quartz .. .. .	6.5
Quartz + anthracite .. .. .	1.1
Quartz + steam coal .. .. .	5.6
<i>Removal of Silica from Solution</i>	
(10 ml. ascitic fluid, containing 13 mg. SiO <sub>2</sub> /100 ml., shaken with 0.2 g. coal dust at 37° C. for 1 week)	
Anthracite .. .. .	10.9
Steam coal .. .. .	12.5
Peat .. .. .	12.8

there be in the organic matter. Peat, therefore, should contain more than bituminous or steam coal, and anthracite least of all; thus, if any depressant action was exerted on the dissolution of silica it should be greatest with peat. This expectation was not realized. Peat and steam coal had almost no effect, but anthracite, surprisingly enough, depressed

TABLE XXXI

*Depression of Silica Solubility by "Clean" Coal Dusts*

(0.2 g. quartz shaken with 1 g. powdered coal for 48 hours at 37° C.)

	mg. SiO <sub>2</sub> per 100 ml.	
	Ringer's solution.	Ascitic fluid.
Quartz .. .. .	6.5	6.3
Quartz + A1 anthracite .. .. .	1.8	1.7
Quartz + A2 anthracite .. .. .	2.2	2.2
Quartz + A3 anthracite .. .. .	2.2	2.3
Quartz + S4 steam coal .. .. .	5.4	5.7
Quartz + B2 bituminous .. .. .	5.5	5.0
Quartz + B3 bituminous .. .. .	4.7	3.9

TABLE XXXII

*Effect of Graded Amounts of "Clean" Coals on Quartz Solubility  
(0.2 g. quartz shaken with Xg. powdered coal in 10 ml. buffered Ringer's solution  
for 60 hours at 37° C.)*

	mg. SiO <sub>2</sub> dissolved per 100 ml.	
	(A1 anthracite.)	(A3 anthracite.)
Quartz alone .. .. .	5.9	5.8
Quartz + 0.2 g. coal dust .. .. .	5.2	3.2
Quartz + 0.5 g. coal dust .. .. .	3.2	1.6
Quartz + 1.0 g. coal dust .. .. .	1.1	0.9

the amount of silica in solution markedly. As this was a very pure sample of anthracite coal, it appeared possible that there might be something about the anthracite substance itself which would depress the dissolution of silica. Several "clean" coals were accordingly tested for their effect on the dissolution of quartz and, as will be seen in Table XXXI, it appeared to be an invariable finding that anthracite coal inhibited dissolution strongly, and steam and bituminous coal weakly or not at all. The two anthracites which had the most marked effect were further tested in variable amounts (with a constant amount of quartz) to see if the depression was proportional to the amount of coal present. That this was the case is shown in Table XXXII.

The "clean" coals used in these experiments contained only very small amounts of non-coal mineral matter; that is, their ash contents were very low (about 1 per cent.), and it seemed improbable that these small amounts could be responsible for the observed effect. However, it seemed advisable to try the effect of still cleaner coal, and Mr. Donald Hicks, of the Fuel Research Laboratory, Cardiff, kindly prepared us some specially-treated samples which were subjected to sedimentation in fluids of different specific gravity, whereby the heavier-than-coal mineral matter was largely eliminated. These purified coal specimens had appreciably less ash (about half) than those previously used. Their ability to interfere with the dissolution of quartz had largely disappeared (Table XXXIII).

TABLE XXXIII

*Failure of Specially purified Coal to Depress Quartz Solubility  
(0.2 g. quartz shaken with 1 g. coal powder at 37° C.)*

	mg. SiO <sub>2</sub> dissolved per 100 ml.			
	In Ringer's (72 hour).	In ascitic fluid (48 hour).		
		Exp. 1.	Exp. 2.	Exp. 3
Quartz alone .. .. .	8.7	6.7	6.8	6.5
Quartz + A1 anthracite .. .. .	7.1	6.2	6.7	6.2
Quartz + A3 anthracite .. .. .	5.5	5.6	5.6	5.6
Quartz + S4 steam coal .. .. .	8.9	6.6	6.7	6.4

It must be assumed, therefore, that the coal substance itself cannot affect quartz solubility, and that the only thing in coal which will inhibit the dissolution of silica is its mineral matter. If this be so, then the ash contents of a series of similar coal dusts should affect silica solubility in a progressive way. We were fortunate in obtaining from Mr. Hicks two sets of powdered coals, from an anthracite mine and a bituminous mine respectively, which had been so fractionated by the sedimentation procedure as to yield specimens of graded ash

content. In Fig. 40 the percentage of ash for each specimen is plotted against the extent to which it depressed the solubility of quartz. A direct relationship is obvious.

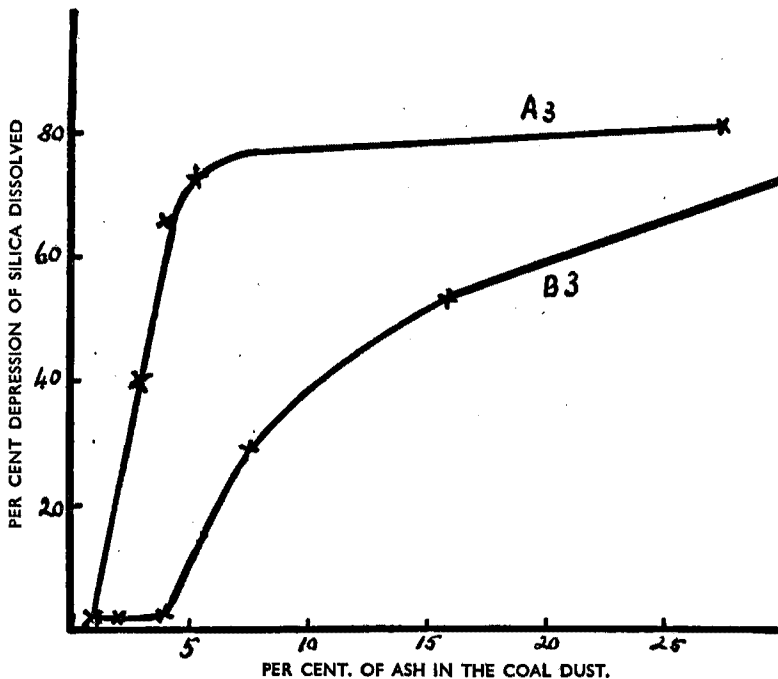


FIG. 40. Relation of the ash content of coal dusts to their ability to depress the dissolution of quartz. 0.2 g. quartz shaken with 1 g. of A3 (anthracite) and B3 (bituminous) coal dust of varying ash content in 10 ml. ascitic fluid for 48 hrs.

*Depression of Silica Solubility by the Ash of "Clean" Coals.*—Several of the "clean" coals were ashed by placing 100 to 200 g. of the powdered material in platinum dishes in an electric muffle furnace maintained at 370° C. By ashing at this low temperature, it was hoped to alter the non-coal mineral matter as little as possible. Complete destruction of the carbonaceous matter took from 24 to 48 hours. Dissolution experiments were then conducted with mixtures of quartz with the powdered coals, and with variable amounts of ashes derived from them. The results are given in Table XXXIV.

TABLE XXXIV

*Depression of Quartz Solubility by the Ash of "Clean" Coal*  
(0.2 g. quartz shaken with 1 g. of powdered coal or x mg. of the ash of the coal in 10 ml. ascitic fluid for 48 hours at 37° C.)

	mg. SiO <sub>2</sub> dissolved per 100 ml.				
	A1 anthracite.	S4 steam coal.	B2 bituminous.	B3 bituminous.	B3 (purified).
Quartz + coal .. ..	2.4	4.4	4.8	4.8	5.2
Quartz + 5 mg. ash ..	2.8	2.5	1.2	1.0	1.0
Quartz + 10 mg. ash ..	2.6	1.5	1.1	0.8	0.9
Quartz + 20 mg. ash ..	2.1	1.5	1.0	0.8	0.9
Quartz + 40 mg. ash ..	1.6	1.5	1.1	0.8	0.8

(Quartz alone yielded 5.4 mg. SiO<sub>2</sub> in solution.)



The ash of the steam and bituminous coals, which of themselves fail to depress silica-solubility, is as effective as that of the anthracite. This fact must mean that the non-coal mineral matter of the non-anthracite coals is somehow bound up or coated by the coal substance, preventing its access to the solvent medium. In the anthracite coals, on the other hand, the minerals appear to be freely accessible to the action of the liquid solvent, and to discharge some of their constituents (e.g., aluminium) into it. As in the case of the shales and the extraneous matter of the coal seams, therefore, the behaviour of the inherent mineral matter of the coal is similar for both anthracite and bituminous. But the coal substance modifies its action, and supplies a contrast between bituminous and anthracite coal in their effect on the solubility of silica.

*Increase in Silica Solubility of Silicate Dusts due to Digestion with Hydrochloric Acid.*—Nearly all the mineral dusts tested in this investigation have been of very low solubility. None has given even approximately as much silica in solution as do quartz and other forms of free silica. The only dust, besides the free silicas, which yielded much more than 1 mg.  $\text{SiO}_2$  per 100 ml. was the Ogofau sericite (Table XXV), which gave 2.9 mg.  $\text{SiO}_2$ . On looking into the history of this specimen, we discovered that it had been treated with hydrochloric acid, as part of the purification procedures to which it had been subjected. It seemed worth while, therefore, to investigate the effect of HCl-treatment on several of the dusts, and particularly on the shales and dusts closely related to Ogofau sericite, such as the fine fraction illites, which are likewise a form of sericite. These almost inert mineral dusts became highly reactive, with one exception, on a simple treatment with dilute hydrochloric acid, either hot or cold, and now yielded large amounts of silicic acid into solution in ascitic fluid,

TABLE XXXV

*Increase in Silica Solubility of Silicate Dusts due to Digestion with Hydrochloric Acid*

(0.2 g. of silicate dust shaken with 10 ml. of Ringer's solution pH 7.4 for 48 hours at 37° C.)

	mg. $\text{SiO}_2$ dissolved per 100 ml.		
	(1) Untreated dust.	(2) Dust treated with cold N HCl.*	(3) Dust treated with hot N HCl.†
<i>Mica illite (sericite)</i> —			
A1 .. .. .	1.8	15.9	—
A3 .. .. .	0.7	—	1.4
S4 .. .. .	1.3	—	11.4
<i>Rock strata</i> —			
A1, 94 LPJ (shale) .. .. .	1.2	6.1	11.8
94 LP2F (shale) .. .. .	1.1	5.7	10.8
A3, A (sandstone) .. .. .	1.3	12.3	13.8
B (clift) .. .. .	1.7	7.0	10.7
C (clod, shale) .. .. .	1.7	7.8	10.6
D (88 per cent. coal) .. .. .	0.7	—	1.1
E (96 per cent. coal) .. .. .	0.7	—	0.7
F (clod, 26 per cent. coal) .. .. .	1.1	—	4.1
G (97 per cent. coal) .. .. .	0.3	—	0.8
H (fireclay) .. .. .	1.2	—	8.6
(Quartz) .. .. .	(4.9)	—	(4.8)

\* Dust shaken with N HCl for 24 hours, separated and washed with water.

† Dust heated with N HCl in a boiling water bath for 2 hours, separated and washed with water.

TABLE XXXVI

Percentage Particle Size for whole Dust, in Samples examined by Dr. T. Bedford and Dr. C. G. Warner (data kindly provided by these workers)

Description of dust.	Percentage of dust particles of size (microns).											No. of particles measured.	Coal particles present (+) or absent (-)	
	Under 0.2	0.2-0.4	0.4-0.8	0.8-1.2	1.2-1.6	1.6-2.0	2.0-2.5	2.5-3.0	3.0-4.0	4.0-5.0	Over 5.0			
<b>Strata of A3 mine—</b>														
A, sandstone .. .. .	18.8	22.1	30.0	16.1	6.9	3.3	1.8	1.0	—	—	—	510	—	
B, clift (shale) .. . . .	12.9	20.3	34.0	17.4	7.8	4.4	1.7	1.3	0.2	—	—	541	—	
C, clod (shale) .. . . .	15.1	25.1	30.3	16.1	7.8	3.4	1.8	0.4	—	—	—	502	—	
D, upper bord coal .. . . .	5.4	8.7	31.5	19.3	12.2	7.8	4.6	2.6	3.7	1.8	2.4	793	+	
E, lower bord coal .. . . .	—	—	—	—	—	—	—	—	—	—	—	—	—	
F, clod (shale) .. . . .	—	—	—	—	—	—	—	—	—	—	—	—	—	
G, coal (working seam)	10.5	9.9	30.0	17.6	12.3	7.0	3.5	2.4	2.6	2.1	2.1	819	+	
H, fireclay .. . . .	—	—	—	—	—	—	—	—	—	—	—	—	—	
<b>Clod (shale) from other mines—</b>														
A1.—94LPJ .. . . .	20.9	31.7	23.9	12.9	6.3	2.5	0.4	0.6	0.6	0.2	—	489	—	
A1.—94LFF2 .. . . .	17.3	27.3	28.2	14.8	6.2	3.1	1.8	0.8	0.6	—	—	486	—	
S4 .. . . .	16.9	30.1	27.7	13.3	6.0	3.0	1.6	0.6	0.8	—	—	498	—	
<b>Dirt from coal seams—</b>														
A3 (34 per cent. ash)	7.4	17.2	22.8	15.2	8.8	7.8	4.4	3.6	4.4	3.8	4.6	500	++	
A1 (33 per cent. ash)	1.4	11.2	16.4	14.4	16.0	14.8	10.0	6.4	5.0	3.0	1.4	500	+++	
A2 (32 per cent. ash)	8.4	19.0	22.6	16.6	12.6	6.4	3.8	4.6	3.2	1.6	1.2	500	+++	
S4 (47 per cent. ash)	4.0	12.0	15.2	16.8	18.8	10.0	8.2	5.6	4.4	2.8	2.2	500	+++	
<b>Air-borne dusts—</b>														
A3.—D .. . . .	10.0	18.6	26.8	15.5	7.3	4.7	3.2	2.3	3.5	4.3	3.8	1,508	+	
A3.—E .. . . .	10.6	14.0	22.4	14.8	10.1	6.7	6.1	3.8	5.1	3.5	2.8	1,531	++	
A3.—I .. . . .	6.6	11.8	22.7	19.5	8.3	8.9	5.9	4.0	6.3	3.5	2.5	1,523	+++	
A3.—J .. . . .	9.7	11.3	24.9	18.5	9.7	8.4	5.5	2.8	4.8	2.2	2.1	1,528	+++	
A3.—2HI4 .. . . .	9.1	11.7	23.4	18.5	9.8	7.9	5.1	3.2	4.9	3.2	2.2	1,534	+++	
A1.—2M1 .. . . .	12.1	13.4	26.1	16.7	10.0	4.9	4.1	4.6	3.8	2.8	1.5	789	+++	
S4.—1U1 .. . . .	10.3	13.5	31.6	17.4	9.2	4.7	3.9	2.7	3.0	2.5	1.2	1,024	+++	
B2.—1C1 .. . . .	9.8	12.4	23.1	18.5	12.0	8.4	4.4	3.3	3.6	2.4	2.1	—	—	
B3.—1B1 .. . . .	8.2	8.5	21.5	18.6	17.7	10.3	4.8	2.7	4.0	2.3	1.7	—	—	
A3.—91US28 .. . . .	7.6	6.6	27.8	20.6	12.7	7.7	4.3	2.7	3.5	2.3	2.2	820	++	
A1.—94US10 .. . . .	8.8	11.4	30.0	21.0	10.3	6.0	3.9	2.5	2.0	2.2	1.9	958	+++	
A2.—95US10 .. . . .	9.0	9.8	29.4	18.6	12.0	8.6	4.1	2.7	2.7	1.7	1.4	778	+++	
S4.—83US10 .. . . .	8.6	8.7	30.6	21.0	12.5	6.0	4.7	2.9	2.1	2.1	0.8	898	+	
<b>Isolated fractions from mine dusts—</b>														
A3. Fine fractions (illite)	(all under 1 $\mu$ ).													
A1. Fine fractions (illite)	(all under 1 $\mu$ ).													
S4. Fine fractions (illite)	(all under 1 $\mu$ ).													
<b>Pure minerals—</b>														
Quartz—Brazil .. . . .	15.4	31.2	25.3	14.9	6.1	3.6	2.4	0.4	0.6	0.1	—	1,000	—	
Quartz—Ontario .. . . .	11.9	19.5	24.3	16.8	8.7	8.2	4.2	2.5	2.2	1.2	0.5	995	—	
Kaolin .. . . .	12.9	27.8	32.2	12.0	7.7	3.6	2.3	0.8	0.5	0.2	—	614	—	
Ogofau sericite .. . . .	4.4	12.2	14.0	20.8	14.8	8.0	4.0	7.0	6.2	3.6	5.0	500	—	
Biotite .. . . .	13.0	18.8	29.5	16.7	6.6	5.4	3.4	1.0	2.3	1.9	1.3	1,025	—	

Ringer's and other solvents. This extraordinary effect may be connected with a deterioration of the crystal lattice structure of the outer layers of the mineral, whereby the atoms of silicon become more exposed or easily detached, and hence pass more readily into a solvent medium in which the mineral is suspended. Many of the dusts have been treated in this way, and representative results are set out in Table XXXV. Although it is not clear that the finding may have a bearing on human pneumokoniosis, it certainly complicates much of the animal experimentation, which has frequently been carried out with powdered specimens of rock subjected to hydrochloric acid treatment. Comment is made in the Discussion on some of the results so obtained by ourselves and by other workers.

### DISCUSSION

Classical silicosis—as seen, for example, in the goldminers of the Rand and in sandblasters—is comparatively rare in the coalminers of South Wales. Simple silicotic nodulation is confined to workers in sandstone—"hard-headers"—who breathe a highly siliceous rock dust, and is not encountered amongst the colliers. These men breathe a dust which consists largely of coal, with considerable shale, and not much free silica. Their lung disease is characterized by an extreme chronicity, and the lesions appear to be either an arrested form of silicosis, in which the earlier type of fibrous change in the tissues due to reticulin-formation is confirmed and established, or a massive fibrosis of confluent and conglomerate character, produced without much distinct nodulation of the discrete type.

Elsewhere in this Report (King and Nagelschmidt, p. 18) we have speculated on the possibility of this type of pulmonary fibrosis being a "low-grade silica effect", due to a mild toxic action of the slowly dissolving silica in the tissues, retarded and confused by the presence of much depressant and diluent dust. We have also begged the question whether some modified mechanical theory of pneumokoniosis ought to be considered. The concentrations of dust found in the lungs of these coal-workers are enormous—so large, in fact, as to raise some doubt as to the ability of alveolar tissue to function normally in the presence of so much foreign matter. The combination of an ever-present, if mildly toxic environment, with a mechanical clogging of the lymph channels and glands, and an interference with alveolar function by the excess of dust, may not be an unlikely hypothesis to account for the peculiar changes in the lung and the rendering of so much of its tissue non-functioning.

The low solubility of these South Wales dusts would seem to imply a low toxicity, but we know that many of them are pathogenic, and some highly toxic. This seeming conflict of evidence may, however, be more apparent than real. It may, indeed, be due solely to the inadequacy of our experiments. The low-solubility dusts of known pathogenicity, i.e., the sandstones, all contain considerable proportions of free silica. Free silica by itself is fairly soluble in the body fluids, and while the mixtures of free silica with other minerals in these sandstones do not appear to be so soluble, their low *in vitro* solubilities may not accurately reflect the state of affairs in the lungs. The low solubilities may probably be related to the release, from other components of the dust, of aluminium, which may form a protective coating over the free silica particles. Within the alveolar spaces, and in the lymph tissues, the releasable aluminium may, however, eventually disappear from these sandstone dusts, and from the particles of free silica, leaving them finally exposed to the inter- and intracellular fluid. Thus there may be produced in the lung mineral material from which all the releasable aluminium has been leached, leaving it more potentially pathogenic than it was at the time of its inhalation. Mineral dusts containing higher proportions of aluminous components should be more inert, even after long residence in the lungs; and this seems to be the case, since it is recognized

that the shales are probably less dangerous to health than the sandstones. The animal experiments showed the A3 sandstone roof to yield a highly pathogenic dust, and the shales to be almost inert. The chief difference between these mineral powders seemed to be a higher proportion of free silica (quartz) in the A stratum (sandstone) than in the clift (B) or clod (C). The failure to reveal any significant difference in their behaviour in *in vitro* solubility studies can, it seems to us, only be reconciled with current theories of silicosis by means of such a postulate as that given above. In future studies, it is hoped to test the idea of there being less total releasable aluminium in the sandstones than in the shales, and more soluble silica finally to be obtained, by means of more protracted experiments, in which the different dusts will be subjected to a prolonged leaching action by constantly renewed solvent.

In connexion with the great difference in the severity and incidence of pneumokoniosis between the anthracite and bituminous fields, only one fact of possible significance has emerged. Powdered anthracite coal, and the air-borne dusts from anthracite mines, depress quartz solubility markedly; steam coal and bituminous very much less. This difference appears to be due to some effect by the coal substance itself, in the cases of steam and bituminous coal, on the aluminous mineral matter contained in it. When freed from the coal substance, the mineral matter of the bituminous dusts is as effective in depressing the dissolution of quartz as is that of the anthracite dusts. The anthracite coal substance, on the other hand, has no effect on its contained mineral matter, which is free to exert its influence. Can it be that the bituminous and steam coals likewise have a protective action on the quartz dust contained in them or with which they become mixed in the air-borne dusts of the mines? Although we have no direct evidence of such an effect, it seems possible on the grounds of analogy. If the bituminous or steam coal when suspended in a fluid is able to prevent its contained aluminous mineral matter from acting on powdered quartz added to it, then it may conceivably also prevent its contained free silica from acting on the tissues with which it comes into contact.\* Experiments to test a possible difference between the effects of anthracite and of bituminous coals on powdered quartz mixed with them have failed to reveal any difference in the lesions produced in animal lungs. Both mixtures resulted in massive confluent fibrosis. Possibly some more intimate mixture of a free silica with the coal, whereby the silica was actively adsorbed by the coal substance, or at least closely adhered to it, might yield evidence of some difference in action as between anthracite and bituminous coals. Further work will be directed to this end.

Brammall and Leech (1937) have shown that dust-fine minerals are very susceptible to the action of dilute hydrochloric acid, solutions of the alkali chlorides, and even water. Thereby a hydrolysis of the surface layers of the particles takes place, and hydroxides of aluminium, ferric and ferrous iron, and some magnesia appear in solution. How greatly a preliminary treatment of a mineral dust may alter its properties is well shown by the figures included in Table XXXV. It is customary to "clean" powdered specimens of rock by extracting them with hot or cold hydrochloric acid and/or sodium hydroxide solution. This results in the elimination of various common impurities and contaminants, e.g., carbonates, pyrites, amorphous silica and alumina, and it no doubt produces a more satisfactory sample, from the standpoint of the analyst and crystallographer, than the parent material. But investigations on such specimens cannot yield information which accurately represents either the true properties of the powdered minerals in a solvent or their behaviour in

\* In this connexion it may be significant that it is generally more difficult to remove the mineral matter from bituminous coals than it is from anthracite, and that the mineral matter of the former is more intimately mixed with the coal substance than it is in the latter (Hicks & Nagelschmidt, 1943, *M.R.C. Special Report Series*, No. 244).

the lungs. Briscoe *et al.* (1937) have brought evidence to suggest that when a rock is fractured to produce a dust, and new particle faces are formed, an immediate hydration takes place at the new faces, penetrating to a depth of something like  $0.2\mu$  and thereby altering the properties of the specimen in respect of its dissolution behaviour. A much more drastic action appears to take place when powdered silicates are treated with strong mineral acids. We have suggested that the acid may alter the crystal lattice structure of the mineral in its outer layers, and it is possible that this alteration penetrates to at least the depth suggested by Briscoe *et al.* for the action of water or of its vapour in the air. The effect will thus be greatest with very finely divided samples, and while it may not appreciably change the analytical figures or the crystal properties of either the gross or powdered specimen, it will certainly change the solubility characteristics of the latter. The direction in which the change takes place will obviously be in that of a greater apparent solubility for the silicates, where the position of the silicon atoms in the crystal lattice, normally partially protected by the atoms of the associated metallic elements, may become exposed through deterioration of the crystal structure under the action of the strong acid. The metallic elements may even be lost by solution in the acid medium, leaving a siliceous residue behind, as has been pointed out by Francis (1945) in the case of asbestos fibres. Although such effects may be only local, and may not affect the mass properties of the specimen, they certainly invalidate the application of results obtained on such material to the problem of silicosis. This is more the pity since it means that the purest and best characterized specimens, as regards analysis, particle-size, etc., are likely to yield the least reliable results for the biological problem; and it is necessary, therefore, to use dusts, for this purpose, which approximate as closely as possible to the actual material breathed into the lung. This organ shows little selectivity in the dust it admits, apart from eliminating the grosser particles, and the best *in vitro* condition to simulate the state of affairs within the lung is a high concentration of untreated dust, and an overwhelming proportion of exceedingly small particles.

This question of the sizing of powdered minerals for solubility studies has exercised many workers, and it has been repeatedly pointed out that it is desirable to use materials of comparable size for the purpose of studying their comparative solubilities. This obvious truth has led to painstaking and commendable efforts to isolate fractions of comparable size by sedimentation (centrifuging and normal gravity settling) and other procedures. But, unfortunately, the lengthy processes of purification and isolation have often involved treatment with solutions of strong chemicals which would appear to have so altered at least some of the minerals under study as to render the solubility figures obtained on them of little value.

Much has been written about the possible relation to lung disease of sericite, originally suggested by Jones (1933) as a cause of silicosis, and many experimental studies, involving both solubility tests and animal inoculations, have been made to test this hypothesis. Sericite is a hydrated potassium aluminium silicate, and results with it may be taken as being typical of those obtained with the silicates which have been examined in regard to their solubilities and possible relation to lung disease. Emmons and Wilcox (1937), and others have reported higher dissolution figures for sericite than were obtained with quartz; and these authors appear to have treated their silicate dusts with hydrochloric acid as part of the purification procedure. Denny *et al.* (1937), Briscoe *et al.* (1937), Whitehouse (1938), King, McGeorge and Roman (1938), Lucas and Dolan (1939), Robertson, Simson and Strachan (1937), on the other hand, have found a lower dissolution of silicic acid from sericite and some other silicates (not asbestos) than from quartz, flint or other free silicas. These workers have used either freshly formed, or collected dust, or powdered minerals,

subjected to no other treatment than sieving or water sedimentation, and it seems, therefore, that the high results reported for the dissolution of silicic acid from sericite by some authors may have their cause in the treatment given the dust before testing it.

The findings of Kitto and Patterson (1942) call for special mention, because these authors appear to have used no strong acid or base in the preparation of their dust samples, and yet obtained higher results for the dissolution of muscovite and orthoclase than of quartz. The unusual course is taken in this communication of reporting the results in percentages of the mineral specimen dissolved, and not in terms of the silicic acid appearing in solution. It is consequently difficult to compare the results with those obtained by other workers, except on a comparison (of quartz with, e.g., mica) basis. The percentage of solid dissolved is, of course, not necessarily a criterion of solubility. As long as the amount of solid phase is sufficient to saturate the liquid taken, the same solubility figure will be obtained, but the percentage of the solid dissolved may be vastly different. We assume, however, that this elementary objection does not apply to the results given by Kitto and Patterson, and that they did, in fact, find more silicic acid to go into solution from muscovite than from quartz. But they do not state that the same concentrations of dust in the liquid were used for the different minerals; nor show what the concentrations of dusts were. Discrepancies between their results and those reported here, and by other workers cited, are probably explicable on the basis of their having striven for a comparative figure at a lower concentration of dust than those used by ourselves and others. We have wished to obtain maximum figures for the silica dissolved from various mineral dusts and have accordingly used large concentrations of mineral dust more akin to those found in the lungs of workers.

Elsewhere, (King, 1937), we have criticised solubility results obtained on solutions from which the solid dust had possibly been imperfectly separated. While it seems improbable that the results of Kitto and Patterson should have been influenced by such a complication, it is perhaps worth pointing out that the description given of their means for separating solid from liquid leaves open the possibility that there may have been very fine particles of dust left in their unfiltered centrifugates; and that the acid molybdate used for determining the dissolved silica has a perceptible solvent action on finely divided siliceous particles.

To their criticism that samples of mine dust, used by ourselves and others in previous investigations, were not of strictly uniform size distribution, we would reply that although this is certainly the case, what error there was in this respect was in favour of the muscovite and shale dusts. Mica (muscovite), for instance, powders more easily and more finely than quartz, and yet the results obtained have yielded lower silica dissolution figures for the extremely fine dust of the shales and separated "fine fraction" micas than for the somewhat more coarsely powdered quartz.

Animal investigations with acid-treated mineral dusts are probably open to the same criticisms as those made against solubility experiments. In terms of chemical theories of pneumokoniosis, any alteration in the chemical behaviour of a mineral dust must change its behaviour in an animal tissue and alter its effect produced on the tissue. Changes produced in mineral dusts which increase the silica they yield into solution will possibly increase their toxic action on alveolar tissue. Particularly in acute experiments of the type where massive concentrations of a dust are introduced into a tissue by an inoculation technique, the large concentrations of silicic acid, to be promptly produced in the fluids bathing the particles in the alveolar spaces and lymphatics, cannot but have a pronounced effect on the tissues, and may well account for the fibrosis produced in the experiments with Ogofau sericite recorded in this Report (p. 55), and in the other experiments described in the literature where sericite and other silicates have been found to produce fibrous reactions similar to those obtained with quartz and flint. Experiments now in progress with acid-treated shales and micaceous fractions should shed further light on this interesting complication of the experimental silicosis problem.

### SUMMARY

(1) Theories of silicosis have been reviewed, and the salient points in favour of those currently held have been noted. At the same time, shortcomings and deficiencies of present theories have been pointed out, and attention drawn to certain aspects of silicosis and pneumokoniosis which they fail to explain.

(2) Detailed studies have been made of the solubilities of South Wales mine dusts, and other control dusts; and the various factors which affect the liberation of silicic acid, such as method of preparation, ageing of the dust, ratio of solid to liquid,  $pH$ , different buffer mixtures, mineral, coal and aluminium content, have been investigated.

(3) All the South Wales dusts studied showed low silica solubilities, i.e., they released much less silicic acid into solution than did quartz and other forms of free silica under identical conditions.

(4) The admixture of shale and other dusts with powdered quartz caused a marked depression of the solubility of the latter. This effect appeared to be related to the release of aluminium from the aluminous components of the shales to the quartz particles, over which it formed a protective coating which could be stained with aurine. The amount of aluminium which could be released in this way, and the extent to which a given dust would depress the solubility of quartz, were not directly related to the total aluminium content of the sample.

(5) In addition to the depression of silica solubility by the stone dusts, a marked depressant effect was produced by samples of "clean" coal, but this was proved to be due to the contained mineral matter (the ash) and not to the coal substance itself.

(6) Anthracite coals depressed the silica solubility of quartz markedly, steam and bituminous coals very much less. The ash of all coals was equally effective. The aluminous (and siliceous) components of steam and bituminous coal must, therefore, be much more closely bound to the coal substance than is the case in anthracite coal. The possible relation of this finding to the perplexing problem of the great difference in incidence of severe pneumokoniosis as between the anthracite and bituminous coal fields has been commented upon.

(7) A large increase has been observed in the amount of silicic acid which can be dissolved from specimens of shale and micaceous concentrates after they have been treated with hydrochloric acid. The possibility that dusts which are normally almost inert in respect of solubility and pathogenicity, may by such treatment be changed into active dusts, has been discussed.

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