16. THE BIOCHEMISTRY OF SILICIC ACID

9. ISOLATION AND IDENTIFICATION OF MINERALS IN LUNG RESIDUES AND AIR-BORNE DUSTS FROM COAL MINES

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(Received 30 December 1940)

THE aetiology of the occupational lung affections described as silicosis, anthracosilicosis or more generally as pneumoconiosis is incompletely understood and a close comparison between the composition of the inhaled dusts and the minerals found in the affected lungs may be a useful preliminary to further knowledge. As most of the mineral particles in the lungs are below 5μ in diameter, identification with the petrological microscope is uncertain and X-ray diffraction apparatus becomes a necessary aid to the microscope. The qualitative knowledge of the minerals present, obtained from X-ray diffraction data, can be implemented by calculation of the minerals based on chemical or spectroscopic analysis.

It is intended in the present communication briefly to describe the methods used for the isolation of minerals from the lung tissue and for the X-ray diffraction analysis of these residues. The removal of coal and of acid-soluble material from air-borne dust from coal mines is also described, and data for a lung residue and an air-borne dust are presented. The chief minerals so far identified in this work are quartz, a member of the mica group and a member of the kaolin group.

Preparation of lung residues

Several methods have been proposed for preparing lung residues for X-ray diffraction work, and in some cases the same residues have been used for microscopic, chemical or spectroscopic analysis.

(1) *Simple drying of formalin-hardened lung tissue.* This method has been used by Sweany *et al.* [1938]: it has the advantage that the minerals are less altered than in any other method, but they are very much diluted by organic tissue and the resulting diagrams are poor (cf. Pl. 1A).

(2) *Destruction of organic material by digestion with acid.* HNO_3 or other acid mixtures have been employed, usually followed by ignition at $600\text{--}700^\circ$ [Jones, 1933; Kahane & Antoine, 1936; Jephcott *et al.* 1938; Burke & Kerr, 1938]. It gives a residue well suited for X-ray diffraction analysis, but it is always possible that minerals have been destroyed or altered by the heat treatment or by the acid attack. Burke & Kerr give evidence that even such a stable mineral as sericite shows after the treatment an X-ray diagram in which the lines are displaced against the lines of the untreated mineral. Any kaolinite present in the lung residues examined by the above investigators would have been destroyed by the heat treatment alone, as this mineral is destroyed by heating above 510° .

(3) *Destruction of organic material by enzymes.* Hicks *et al.* [1937] used this method. It is, however, slow, only part of the tissue can be removed and it is not easily applied to formalin-hardened tissue.

(4) *Removal of organic material by H_2O_2 .* This method, described by Sundius *et al.* [1936] and recently used by Gärtner [1939], is in our opinion the best available, as it avoids the action of high temperatures and strong acids. As modified for the purposes of the present investigation, there are three stages in the treatment:

1. Removal of fatty material from powdered oven-dry lung with acetone.
2. Digestion with H_2O_2 .
3. Extraction of the residue by acid or buffer solution.

The object of the third stage is to remove the endogenous inorganic constituents of the lung, mainly Ca, Fe, K, Na and P, as completely as possible with minimum alteration of the minerals, which form the dust in the lung. It may in some cases be impossible to attain both objectives quantitatively, but a fairly good separation can be made with repeated extraction with hot disodium hydrogen citrate at pH 6, or by brief treatment with cold *N* HCl. We have found that even prolonged treatment with *N* HCl or 2*N* HCl does not affect the appearance of X-ray diffraction diagrams of lung residues or mine dusts, as far as the diffraction lines of quartz or silicates of the kaolin and mica groups are concerned. That some small destruction of the quartz or more likely of the layer lattice silicates does take place is shown by the fact that SiO_2 is found in solution in the HCl extract, but this represents only a few per cent of the total silica in the dust or lung residue and would therefore not be reflected in X-ray diagrams with our present technique. Some results illustrating the extent of decomposition of mineral dusts by cold *N* HCl are shown in Table 1. With lung residues, decomposition and losses with cold *N* HCl and with hot disodium hydrogen citrate solution (pH 6) were slight. The amounts of SiO_2 in solution in the combined extraction and wash fluids have never exceeded a few mg., and much of this is derived from the amorphous silica of the lung tissue. The amounts of dissolved SiO_2 found in the cold HCl extracts have not been greater than those in the hot citrate extracts.

Table 1. *Treatment of powdered minerals and of air-borne dusts from coal mines with HCl*

1 g. powder shaken with 50 ml. *N* HCl at room temperature for 5 hr.

	mg. SiO_2 dissolved	Decomposition %*
Powdered minerals:		
Quartz	0.33	0.03
Kaolin	0.34	0.07
Mica sericite (Ohio)	1.81	0.45
Mica illite (S. Wales)	4.65	1.0
Air-borne dusts:		
2H2 (7.2% SiO_2)	1.26	1.8
2M1 (6.9% SiO_2)	1.22	1.8
1S1 (2.9% SiO_2)	0.48	1.7
1U1 (6.4% SiO_2)	0.55	0.9

* $100 \times \text{Dissolved } SiO_2 / \text{Total } SiO_2 \text{ per 1 g. powder.}$

The lung residues obtained by the H_2O_2 method may, however, contain large amounts of carbon (up to 80 %), and the same applies to dusts collected in coal mines. Such carbon can be removed by prolonged heating of the samples to

380° in an electric muffle. This temperature is sufficiently high to oxidize the carbon in 40–80 hr. and at the same time low enough to avoid destruction of the kaolinite lattice. Pyrites and other sulphides and hydroxides which may be present in air-borne dusts would be oxidized or dehydrated by this treatment, and they should either be centrifuged out of the dust by means of heavy liquids before heating, or identified by the X-ray diagrams of the corresponding oxides, which would be present in the heated but not in the unheated material.

X-ray diffraction methods

In addition to taking X-ray powder diagrams in a cylindrical camera, we have used the aggregate method which has recently been described [Nagelschmidt, 1939] in its application to soil colloids. This method is specially suitable for the identification of layer lattice silicates such as mica, kaolinite, chlorite and montmorillonite in mixtures with each other. Ordered aggregates are made by slow evaporation of a suspension of the sample to be examined, and the aggregates are mounted similarly to single crystals in an X-ray spectrometer. The particles in the aggregate are oriented with their cleavage planes parallel to the plane of sedimentation and a set of good X-ray reflexions from the basal planes is therefore obtained. These basal reflexions are the main X-ray diffraction characteristics for the different layer lattice silicates. Kaolinite has strong basal reflexions at 7.14 and 3.57 Å., and mica has strong reflexions at 10 and 3.34 Å. and a weaker one at 5 Å. When aggregates of mixtures of these two minerals are heated to 510°, only the mica reflexions remain; the kaolinite reflexions are absent, as kaolinite is destroyed by heating to 500°. Chlorite shows strong reflexions at 14.1, 7.05, 4.70 and 3.52 Å. and these reflexions are still present in aggregates heated to 510°. The aggregate method is used in addition to, but not as a substitute for, the powder method. Powder and aggregate diagrams of a lung residue and a mine dust are shown in Pl. 1.

X-ray diffraction diagrams of lung residues and mine dusts indicate qualitatively the presence of different minerals if these minerals occur in sufficient amounts. Whether and, if so, how far both the powder and aggregate methods can be developed quantitatively is at present unknown. The quantitative estimation of quartz in mine dusts has been described by Clark & Reynolds [1936], but as it is based on the intensity of the 3.34 Å. reflexion of quartz it cannot be used in the presence of mica, which gives a strong reflexion for the same spacing. The method can, however, be modified, so as to be applicable to mixtures containing quartz and mica.

Preparation details

The lung is dried at 105° and reduced to a fine powder. 20 g. of the dried substance are extracted in a Soxhlet overnight with acetone to remove fatty material. The extraction thimble with its contents is dried in an oven; the contents of the thimble are knocked into a 2 l. pyrex beaker; 30 % H_2O_2 is added to a depth of about 1 in., and the mixture is carefully warmed on a water bath and stirred with a glass rod. If the froth tends to rise to the rim of the beaker, it can be whipped down with the stirring rod and if necessary octyl alcohol is added. As the frothing subsides, and as the liquid evaporates, more H_2O_2 is added. The depth of the liquid in the beaker should not exceed about 1 in., otherwise the mixture will tend to froth over. The additions of H_2O_2 , with subsequent evaporations almost to dryness, should be continued until the additions no longer cause frothing. At this point the destruction of the lung tissue seems to be complete and any black material remaining is probably coal dust.

The whole process, in our experience, takes about a week. The contents are brought nearly to dryness each evening. Each morning, and as often during the day as the mixture comes to dryness, more H_2O_2 is added.

The final residue consists of the inorganic salts from the lung tissue plus the dust. The former are brought into solution by extracting the mixture with acid. We have used *N* HCl with 15 min. vigorous shaking at room temperature, and hot *M*/10 citrate buffer of *pH* 6 with continuous stirring for 1 hr. On allowing the mixture to settle, the supernatant liquid is seen to contain much of the iron in solution. Separation of the solid from the liquid can be accomplished by gravity filtration through a fine paper (a hardened filter paper seems to retain the dust particles), or by prolonged centrifuging. We have found it convenient to transfer the residue, with washing, to 250 ml. centrifuge bottles, and to use 200 ml. portions of liquid for each extraction. Each separation is carried out by centrifuging for 1 hr. at 3500 r.p.m.

The residues are re-extracted several times to remove all salts. Iron is the most difficult of the tissue constituents to eliminate from the residue, and it is usually necessary to extract the centrifuged deposits three or four times with cold HCl or hot citrate buffer. The presence of iron in the extraction fluids may be tested for by treating a sample with acetic acid, sodium acetate and ferrocyanide.

The filtrate or supernatant fluid should be checked for the absence of dust particles by microscopic examination, and by colorimetric analysis for total SiO_2 [King, 1939]. The presence of appreciable numbers of dust particles will indicate the necessity for more prolonged centrifuging. With the residues we have handled, 1 hr. spinning appears to have been sufficient. The presence of non-particulate SiO_2 , i.e. dissolved SiO_2 , in any quantity indicates a decomposition of the siliceous matter by the extraction fluids. The residue is finally washed once with water and once with alcohol and is dried in an air oven.

Results

As an example, we give full details for one lung residue and one mine dust. The lung was kindly supplied by Dr P. D'Arcy Hart and the mine dust was collected by Dr P. F. Holt, working with Prof. H. V. A. Briscoe. The occupational history of the case from which the lung residue was taken will be given elsewhere, in conjunction with the results obtained in the study of a large group of workers.

Lung residue. The lung residue prepared in this way lost 12% of the dry weight on prolonged heating to 380°. Powder and aggregate X-ray diffraction diagrams of the 380° ash taken with unfiltered iron K radiation are shown in Pl. 1, B and E. A powder diagram of the untreated oven-dried powdered lung tissue is shown in Pl. 1, A. Table 2 gives the interplanar spacings and intensities of diffraction lines of the 380° ash diagram, and the interpretation in terms of quartz, mica and kaolin. There are many diffraction lines of quartz which do not coincide with other lines in the diagram, but only two lines, the basal reflexions, which can be ascribed to kaolin alone. It is not possible to distinguish under these conditions which member of the kaolin group it is; it can only be said that a member of the group is present. The kaolin group contains the minerals kaolinite, nacrite, dickite, anauxite and halloysite. No other mineral is known to give strong basal reflexions at 7.1 and 3.55 Å. spacings, which disappear if the sample is heated to 510°. For the mica it is likewise not possible to determine which member of the mica group is present. The diagram does not seem to correspond to well-crystallized muscovite or hydromuscovite, but rather to the

type of mica found in shales and soil colloids. The term 'illite' [Grim *et al.* 1937] has been proposed for mica of this type, but samples described as sericite or secondary muscovite may refer to similar material.

Table 2. *X-ray data for lung residue, 380° ash*

d = interplanar spacings, Int. = intensities of diffraction lines, v.s. = very strong, s. = strong, m. = medium, w. = weak, v.w. = very weak. Q. = quartz, Mi. = a member of the mica group, Ka. = a member of the kaolin group.

} denotes edges of a band or two lines of different intensity which are not clearly separated.

No.	d	Int.	Mineral	No.	d	Int.	Mineral
1	10.1	s.	Mi.	13	1.815	s.	Q.
2	7.1	s.	Ka.	14	1.675}	m.	Mi., Ka., Q.
3	5.0	w.	Mi.	15	1.644}	m.	Q.
4	4.48}	s.	Mi., Ka.	16	1.539}	s.	Mi., Ka.
5	4.23}	m.	Q.	17	1.508}	s.	Q.
6	3.56}	m.	Ka.	18	1.488}	w.	Mi., Ka.
7	3.35}	v.s.	Q., Mi.	19	1.382}	m.	Q.
8	3.31}	s.	Mi., Ka.	20	1.372}	m.	Q.
9	2.58}	v.w.	Mi., Q.	21	1.294}	w.	Mi., Ka.
10	2.54}	v.w.	Ka., Q., Mi.	22	1.280}	m.	Q.
11	2.46}	m.	Q., Mi.		1.200}	w.	Q.
12	2.45}	m.	Mi., Ka., Q.		1.180}	s.	Q.
	2.18}				1.151}		
	2.131}				1.076}		
	2.003}						
	1.972}						

Table 3 gives the chemical analysis of the lung residue carried out on the 380° ash. The water lost below 105° shown in Table 3 has been reabsorbed from the air by the ash. It will be seen that SiO_2 , Al_2O_3 , K_2O , and water above 105°, account for 94 % of the residue, and these four are the main chemical constituents of a mixture of quartz, muscovite and kaolin. It is possible to calculate the relative amounts of the three minerals in the lung residue if it is assumed that all the SiO_2 , Al_2O_3 and K_2O are present in these minerals, and if the composition of the muscovite and of the kaolin are known. Such calculations with kaolin and muscovite of theoretical composition give for the lung residue: muscovite 26 %, kaolin 46 % and quartz 24 %. The chemical composition of

Table 3. *Chemical analysis of lung residue ashed at 380°*

Analyst: Chemical Laboratories, London

SiO_2	56.9
Al_2O_3	28.3
TiO_2	1.4
Fe_2O_3	1.5
MgO	0.7
CaO	0.5
Na_2O	0.8
K_2O	3.1
H_2O , above 105°	5.4
H_2O , below 105°	1.1
P_2O_5	0.1
	99.8

mica may vary within wide limits, and the mica found in shales and soil colloids usually contains only 4–6 % K_2O , as against muscovite with 11.8 % K_2O . Calculation of the minerals in the lung residue with a mica containing 5 % K_2O , 48 % SiO_2 and 32 % Al_2O_3 , and kaolin of theoretical composition, gives for the

residue a mixture of 62 % mica, 21 % kaolin and 17 % quartz. The X-ray diffraction data indicate only that all three minerals occur as main constituents. Their true relative proportions probably lie between the two sets of percentages given above. If special interest is centred on SiO_2 , it will be seen that less than half of the total SiO_2 shown in the analysis, or less than a quarter of the mineral residue, occurs as quartz.

Microscopic inspection of the 380° ash of the lung residue shows that nearly all particles are well below 2μ in diameter and it does not seem possible to distinguish the different constituents quantitatively by their refractive index or other optical characteristics with microscopic petrological methods.

Mine dust. The untreated mine dust gave a poor X-ray diffraction diagram. After heating the material to constant weight at 380° a residue consisting of 15.1 % of the original dust was obtained. X-ray powder and aggregate diagrams of this residue are shown in Pl. 1, D and F, and a diagram of the same residue after a short treatment with N HCl in Pl. 1, C. Pl. 1, G shows an aggregate diagram, taken with the same specimen as Pl. 1, F, after heating it to 540° for 24 hr. The kaolin lines are absent. Table 4 gives the evaluation of the diagram

Table 4. *X-Ray data for mine dust, 380° ash, after treatment with N HCl*

d = interplanar spacings, Int. = intensities of diffraction lines, v.s. = very strong, s. = strong, m. = medium, w. = weak, v.w. = very weak, Q. = quartz, Mi. = a member of the mica group, Ka. = a member of the kaolin group.

} denotes edges of a band or two lines of different intensity which are not clearly separated.

No.	d	Int.	Mineral	No.	d	Int.	Mineral
1	10.2	m.	Mi.	13	1.540	m.	Q.
2	7.1	v.s.	Ka.	14	1.508	m.	Mi.
3	4.48	v.s.	Ka., Mi.	15	1.493	s.	Ka.
4	3.55	s.	Ka.		1.484		
5	3.35	v.s.	Q., Mi.	16	1.380	s.	Q.
	3.31				1.370		
6	2.58	s.	Ka., Mi.	17	1.318	w.	?
7	2.55			18	1.286	w.	Ka., Mi.
	2.37	v.w.	Mi., Q.	19	1.255	w.	Q.
8	2.33	m.	Ka.		1.236	v.w.	Ka., Mi.
9	2.131	w.	Q., Mi.	20	1.225		
10	1.998	m.	Ka., Mi., Q.	21	1.200	m.	Q.
	1.980			22	1.180	m.	Q.
11	1.816	m.	Q.	23	1.151	w.	Q.
	1.671	s.	Ka., Mi., Q.		1.076	m.	Q.
12	1.642						

shown in Pl. 1, C. It is again only possible to state that quartz and members of the kaolin and mica groups are present. The presence of a line at $14.3 \text{ \AA}$. on aggregate diagrams may possibly indicate minor amounts of chlorite. The diagram before the N HCl treatment shows a few additional diffraction lines indicating dolomite or ankerite. Dolomite is a calcium-magnesium carbonate and ankerite a calcium-magnesium-iron carbonate.

Table 5 gives the chemical analysis of the 380° ash of the mine dust. SiO_2 , Al_2O_3 , K_2O and water above 105° account for 86 % of the ash and most of the remaining Fe, Ca and Mg is present as carbonate, sulphate or oxide, although a proportion of these may form chlorite with some of the SiO_2 and Al_2O_3 . The mineral content has been calculated ignoring chlorite and assuming all SiO_2 , Al_2O_3 and K_2O to be present as quartz, kaolin or muscovite of theoretical composition. The mine dust ash contains according to this calculation 14 % muscovite, 66 % kaolin and 11 % quartz. Assuming a mica containing 5 % K_2O ,

Table 5. *Chemical analysis of mine dust ashed at 380°*

Analyst: Geochemical Laboratories, London

SiO ₂	47.9
Al ₂ O ₃	31.6
TiO ₂	0.8
Fe ₂ O ₃	3.9
MgO	1.4
CaO	3.1
Na ₂ O	1.0
K ₂ O	1.6
H ₂ O, above 105°	4.6
H ₂ O, below 105°	0.7
CO ₂	0.8
SO ₃	2.2
S	0.1
	<hr/> 99.7

48 % SiO₂ and 32 % Al₂O₃ the mine dust would contain: 32 % mica, 54 % kaolin, 8 % quartz. The water above 105° shown in Table 5 is too low to satisfy the amount of lattice hydroxyl present in kaolin of theoretical composition for either of these mixtures, but many kaolin samples contain less than the theoretical amount of water and part of the lattice hydroxyl may have been removed by the initial prolonged heating to 380°.

Free SiO₂ (quartz) [Shaw, 1934] amounted to 12 % of the mine dust ash, in fair agreement with the estimate of 11 % from the calculation with muscovite and kaolin of theoretical composition. We are grateful to Mr A. Shaw for carrying out this determination.

SUMMARY

The isolation of mineral residues from lungs by the H₂O₂ method and the removal of coal from such residues and from air-borne dusts from coal mines by heating to 380° are described. X-ray diffraction methods are used for the identification of minerals in the residues and full data, including chemical analyses, presented for a lung residue and a mine dust. Quartz, a member of the kaolin group and a member of the mica group are the main constituents found in the lung residue and in the air-borne dust.

Grateful acknowledgement is made to Dr A. Brammall for his advice and active interest in this investigation. We are indebted to the Medical Research Council for a grant covering part of the expenses of this investigation.

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X-Ray diffraction diagrams of a lung residue and a mine dust. A-D, Powder diagrams; E-G, aggregate diagrams. A, Oven-dry lung tissue; B, lung residue, 380° ash; C, mine dust, 380° ash after acid washing; D, mine dust, 380° ash before acid washing; E, lung residue, 380° ash; F, mine dust, 380° ash; G, same as F after heating to 520°. For reproduction, intensities in the centres of A-D have been reduced by partial shading.