

ACCUMULATION AND COMPOSITION OF INHALED PARTICULATES IN HUMAN LUNGS

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SUMMARY

The black particulate matters deposited and accumulated in the autopsied human lung of deceased residents of the Tokyo Metropolitan area with no history of lung disease were separated, and their composing elements and substances were identified using several analytical techniques. The origin of lung contamination was examined. The carbon free radicals detected from human lungs were an original finding. Alpha-quartz was identified, carbon element, minerals and trace elements were determined, asbestos fibers were found and a result of the mutagenicity test on deposits was positive. The results observed in most cases were almost identical to the composition of an urban atmosphere. As for the exogenous factors related to the formation of pulmonary lesions, the effect of tobacco smoking cannot be ignored. In our pathohistological study, the observations of pulmonary lesions were found to be related to smoking. In view of the high concentration of element observed therein, it is considered that multiple factors participate in the development of exogenous pulmonary disease due to substances in the environment. These findings may be important in elucidating factors involved in the development of the lung disease due to particle deposition.

INTRODUCTION

The pulmonary anthracosis which has generally been assumed to have little pathological significance has been used as a simple indicator for estimating exogenous lung contamination. In studying the effects of suspended particulate matters in the atmospheric environment, it should be noted that the amount of black dusts deposited in a life time in human lungs depend upon various factors such as age, place of residence, smoking habits, and occupation. Published research reports pertain to anthracosis in the lung of such animals as dog, monkey, pigeon and autopsied human tissues.^{1,2,3} These studies were conducted from the pathological standpoint. However, there has been a need to chemically analyze the composition of black deposits only, because it has been thought that black deposits are mainly composed of inhaled suspended particulate matters in the atmosphere. In a report published⁴ in which Ohta was a co-author, multiple elements analysis was first conducted on anthracosis using spark source mass spectrometry. The ongoing studies^{5,6,7} have concentrated on the establishment of a relatively large base of data obtained by analyzing particulate matters isolated from autopsied lungs not only for element but also for accumulated toxic materials using several analytical techniques.

MATERIALS AND METHODS

Lung Specimens and Pathohistological Findings

The autopsied lung samples used for this study were taken exclusively from people living in the Tokyo Metropolitan area with no history of lung disease. The age of 108 cases ranged from the second decade to the ninth decade. A defined

site of the left upper autopsied lung lobes of these cases was employed, and pathohistological observations were examined.

Separation of Black Deposits from the Lung Tissue

The lung tissue was dissolved in alkaline solution. First, a test was made to determine whether 0.5N NaOH or 0.5N KOH would be satisfactory. Results by these solutions were not so different in regard to dissolve the lung tissue. After weighing the lung tissue which was kept in room temperature after removal from storage at -80°C , they were cut into small pieces and placed in polyethylene bottles with demineralized water to eliminate blood. After repeating this procedure for a few times using high speed centrifugation, 0.5N NaOH which was used for many samples were poured into the bottles. By repeated ultrahigh speed centrifugations at 12,000 rpm and 30,000 rpm, the solid residue was retained. The final residue was then washed using water, ethanol, acetone and finally dried. These black powders were used as samples for analysis.

Deposition Rate and Observation of Particulates Using Scanning Electron Microanalyzer (SEM-EDAX)

Elementary Analysis

1. Determination of elementary content using SEM-EDAX for obtaining general survey of the particulate components
2. Quantitative analysis by neutron activation

The analysis of Mn, V, Al and Ti in 92 samples was completed, and other selected 13 samples were analyzed into trace elements. The samples were irradiated for 30 sec. for short half-life nuclides and for 5 hours for long half-life nuclides at 1.5×10^{12} n/cm² · sec.

3. Determination of carbon content using CHO Elemental Analyzer

In the analysis of total carbon content, CHO Elemental Analyzer was used. A sample measured precisely to 0.3 mg or 1.2 mg was placed in a sample container. Elemental carbon content was measured by combustion at 300°C for 30 min. The volatilized carbon was calculated by subtracting the weight of residue carbon from the total carbon.

Detection of Free Radicals in the Black Particulate Deposited in Lungs Using Electron Spin Resonance (ESR)

Soot, tobacco, other kinds of smoke and products of combustion are serious sources of harmful particulates. Samples of black deposits from the lung which were removed with tweezers without any chemical treatment were lyophilized and approximately 20 mg of each sample was subjected to ESR analysis at room temperature. Solid DPPH was used as standard for the g factor and its benzene solutions was used for the estimation of radical concentration.

Mutagenicity Test for Black Deposits in the Lung

A mutagenicity test for black deposits isolated directly from the lung tissue was examined by the Ames Test. The strain used for this test was *Salmonella typhimurium* TA98 and TA100.

Identification of Crystallized Materials in Deposited Dust

The crystallized material in the black dust which was treated with alkaline solution was identified using X-ray diffraction for 50 cases.

Detection of Asbestos

The asbestos fibers were detected and identified using a transmission electron microscope (TEM) coupled with X-ray microanalyzer for selected samples.

RESULTS AND DISCUSSION

Pathohistological Findings

Some of 108 cases were detected to have pathohistological findings. The main observed findings were chronic bronchiolitis, emphysema and pavement epithelium metaplasia. These cases were found in relating to smoking considerable amount of cigarettes. This is especially true of 9 cases found in this study.

Deposition Rate and Observation of Deposited Particulates

The deposition rate of inhaled dust was positively correlated with age. Correlation factor (*r*) was 0.65 (*n*=95, *p*<0.001). The particle size and shape of collected dust particles from human lungs were observed that an individual particulate was

approximately 0.1 micron in diameter, and many particles had aggregated into clumps.

Elementary Composition of Deposited Particulates and their Accumulation in the Lung

Usually Mg, Al, Si, P, S, K, Ca, Ti and Fe were detected in almost all samples, while Cl and Zn were detected in many samples, those contents were represented in weight percent (wt%). The concentration of Hg, Cr, Fe, Zn, Co, Ag, Sb, Cd and As in 13 specimen's samples were determined by neutron activation analysis. In case of chromium worker, Cr concentration was very high because of exposure to hexavalent chromium. V and Mn in the particulate are considered to originate from artificial sources, such as fuel or combustion, Al and Ti are assumed coming from soil or sand in the natural environmental sources. The concentration of these elements were determined. We attempted to correlate the concentration of element to age. The correlation factor (*r*) of Al was *r*=0.48 (*n*=92, ***), that of V, *r*=0.40 (*n*=91, ***), that of Si, *r*=0.46 (*n*=95, ***), that of Fe, *r*=0.34 (*n*=95, ***). These elements showed a positive correlation to age, that is, they were accumulated in the lung according to increase in age. However, Mn and Ti were not correlated to age. And furthermore, Ca concentration showed a negative correlation to age (*r*=−0.56, *n*=72, ***). The average total carbon content was 55 wt% (*n*=77). The data comparing the total carbon content between smokers and nonsmokers were not discriminating. The average content of elemental carbon was 39 wt% (*n*=39). The volatilized carbon was considered to be organic carbon.

Determination of Free Radicals in Black Deposits

Carbon-centered free radicals were detected in all 21 specimens. Figure 1-a shows an ESR spectrum from specimen A who was 81 year-old woman, where a narrow singlet is seen with a width of 2.7G and a factor of 2.0025. This spectral component is designated R₁. This is more evident in the spectrum obtained with a wider field sweep in Figure 1-b. Such a broad signal apparently arises from inorganic magnetic species in the black deposits. Carbon-centered free radicals are not among those substances commonly expected to be contained in the air dust. Thermolysis or combustion of hydrocarbons is essentially a free radical process accompanying bond cleavage. As a simple comparison, tar and ash from Japanese cigarettes were collected and measured. The observed spectra were the same as that shown in Figure 1. The intensity of R₁ component in each specimen measured by the height of the derivative peaks was obtained.

Mutagenicity Test for Black Deposits

A mutagenicity test which is called the Ames Test was used to determine the black deposits, which were isolated directly from lung tissues, and a small amount of them was used in this test. They were set in the center of an agar plate, the so-called spot test. The strains used for mutagenesis testing were *salmonella typhimurium* TA98 and TA100, S-9(−) and S-9(+). some inhibition was observed, however, His⁺ revertants were not more than the numbers of spontaneous revertants both in S-9(−) and in S-9(+). The result was that one sample was positive in both of TA98 and TA100 to

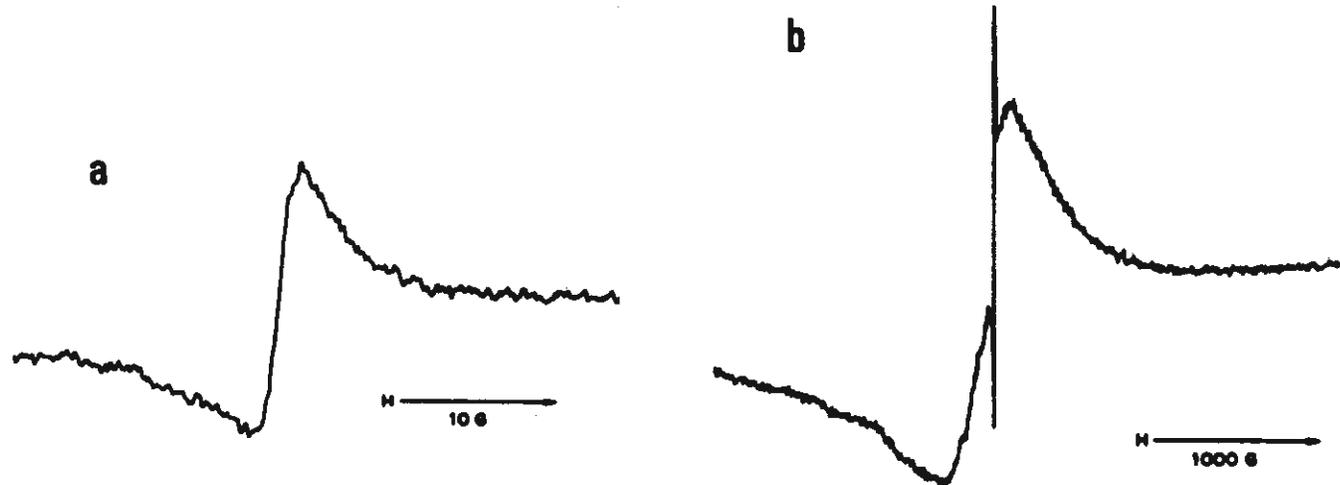


Figure 1-a. ESR spectrum of carbon free radical signal of specimen A who lived in the center of Tokyo for 60 years. Age: 81, Tobacco (-). The signal consists primary of R_1 type radical case. Gain = 4×10^4 . Modulation width = 1G at $g = 2.00$.

Figure 1-b. The whole ESR spectrum of the same specimen taken with a wide magnetic field sweep. Besides R_1 radical (the sharp signal in the center), a very broad absorption due to inorganic magnetic species is observed. Gain = 1×10^4 . Modulation width = 10G.

S-9(-) and S-9(+), 3 samples were probably positive in TA98 only.

Identification of Crystal Structure in the Deposited Particulates

Alpha quartz was detected in 55 samples and crystallized stearate calcium was also detected. In a few samples, talc and ferric hydroxide were detected. Alpha quartz ($\alpha\text{-SiO}_2$) is a natural mineral, originating from soil and rock. They were blown up in the atmosphere. The stearate calcium detected in the lung deposit was produced by chemical procedures with alkaline solution at 40°C for several days.

Detection of Asbestos Fibers in the Black Deposits

Asbestos fibers were detected in three cases among the 10 samples. We studied them using a TEM-XMA to identify asbestos fibers in the lung deposits. Chrysotile fibers were found in specimen B who was 78 year-old medical doctor, tremolite fibers and crocidolite fibers were detected in specimen C who was 65 year-old man. These fibers were qualitatively examined. However, other samples were not detected asbestos fibers.

CONCLUSION

The black dust deposited and accumulated in the human lung were separated and through the identification of their composed elements, crystallized materials, carbon free radicals, asbestos fibers and the mutagenicity test, the origin of lung contamination was examined. The results observed in most

cases were identical to the composition of an urban atmosphere except for several cases, which were depended on their profession. The one case was a hexavalent chromium worker, and others were laborers worked at a industrial factory, public engineering works, construction industry etc. As for the exogenous factors related to the formation of pulmonary lesions, the effect of smoking cannot be ignored. We have detected free radicals in the human lung deposits as an original finding related to smoking and soot. A mutagenicity test for black deposits also was examined and a few cases were positive. Some of these data provide a large information base for future work and will be useful for making a risk evaluation for lung contamination by low exposure to toxic substances.

REFERENCES

1. Shibata, E.: The relation between human lung deposits and air pollution observed to autopsied lungs. *Kyōbu Shikkan*. 7(10):52-57 (1963).
2. Tateishi, R., Morimura, Y. and Hamori, M.: Multiple reactions of smoking and air pollution on the human lung (pathological study). *Nippon Rinshō*. 25:184-192 (1967).
3. Shima, S. and Kato, Y.: *The index of literature of pneumoconiosis*. pp. 158-161, 168-170. Fujita Gakuen University Publication, Japan (1982).
4. Toyohara, K., Shigematsu, A., Hajikano, H., Iwai, K., Aoyama, T., Watanabe, E., Ohta, Y., and Yoshimura, S.: Elementary analysis of dust particulates deposited in human lungs. *Proc. 8th Conference on Radioisotopes. 13-15 November, Tokyo*. 226-228 (1967).
5. Ohta, Y., Inui, M., Shiraishi, H., Tabata, Y. and Wakisaka, I.: Investigation of carbon black dust deposited in human lungs with special reference to influence of the environment. *Proc. VIth World Congress on Air Quality*. 2:181-188 (1983).

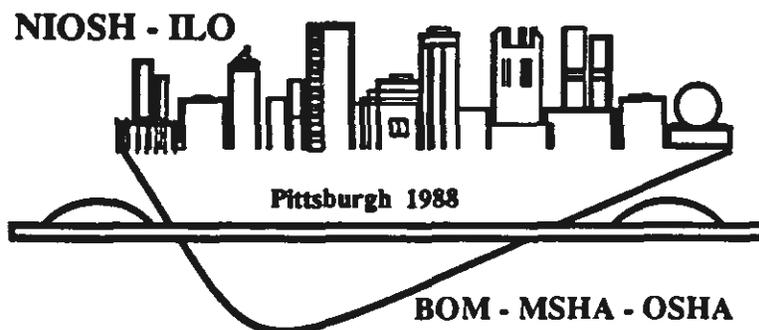
6. Ohta, Y., Inui, M., Shiraishi, H., Matsumoto, M.: Air quality of Tokyo Metropolis evaluated by analysis of human lung deposits. *Internal Medicine Today and Tomorrow*. T. Oda et al. Editors. 106-112. Elsevier Science Pub.(1986).
7. Ohta, Y., Shiraishi, H. and Tabata, Y.: An electron spin resonance

study of free radicals in black dust deposited in human lungs. *Archives of Environmental Health*. 40:279-282 (1985).

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