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Analysis of titanium pigments in human lung tissue

by EGIL M. OPHUS, M.Sc.,¹ LARS RODE, M.D.,² BJØRN GYLSETH, M.Sc.,³ DAVID G. NICHOLSON, Ph.D.,³ and KHALID SAEED, M.Sc.³

OPHUS, E. M., RODE, L., GYLSETH, B., NICHOLSON, D. G. and SAEED, K. Analysis of titanium pigments in human lung tissue. *Scand. j. work environ. & health* 5 (1979) 290—296. Methods are described for the unequivocal identification of mineral deposits in lung tissues obtained during the autopsy of a titanium dioxide-exposed worker who died of an undifferentiated malignant tumor. The methods included scanning and transmission electron microscopy, electron and X-ray diffractometry, and atomic absorption spectroscopy. The presence of considerable amounts of rutile was revealed. Despite this heavy loading, no lung tissue reaction was evident. Within the limits imposed by the period since the first exposure (6 years), this finding lends support to the supposition that the rutile crystal modification of titanium dioxide is biologically inert.

Key words: lung tissue, titanium pigments.

In the process of producing titanium dioxide pigment, workers may be exposed to high dust concentrations, especially during the grinding of the pigment. The presence of large deposits of titanium dioxide pigments in the lungs of workers in titanium dioxide factories has been reported (2, 6, 7).

Titanium dioxide is generally considered to be a harmless substance. Previous evaluations of the biological effects of titanium dioxide pigments in humans and

animals have neglected to differentiate between the rutile and anatase crystal modifications of this compound. Recently, the biological activity of the two modifications has been taken into account in in vitro studies (9).

The aim of the present study was to determine the crystal modifications of the pigments and the quantities of titanium present in different lung lobes of a patient with significant deposits of the pigments and to show how inorganic particles can be identified in lung tissues by various electron beam techniques.

MATERIAL AND METHODS

Lung tissue samples were obtained during the autopsy of a 55-year-old man who died

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of lung metastases from an undifferentiated tumor in the right ileal bone. The patient had been employed for three years in a factory which processed titanium pigments. Initial occupational exposure took place six years prior to death.

Samples for transmission electron microscopy were fixed in glutaraldehyde and osmium tetroxide, dehydrated in ethanol, and embedded in Epon. Thin sections (60–70 nm) were cut with glass knives on a LKB ultramicrotome and stained with uranyl acetate and lead citrate. Semi-thin sections (150 nm) were left unstained for energy dispersive X-ray microanalysis (EDS). Ultrastructural examinations and microanalyses of sections mounted on copper grids were carried out in a Philips 300 transmission electron microscope (TEM) equipped with an EDAX EDS system. During the microanalyses the microscope was operated at an acceleration voltage of 100 kV with a specimen tilt of 48 degrees. Electron diffraction studies were carried out on the same specimens at an acceleration voltage of 200 kV in a JEOL JEM 200 A electron microscope.

Scanning electron microscopy and X-ray microanalysis were performed on dehydrated and critical-point dried lung tissue, as well as on low-temperature ashed samples prepared according to the method described by Gylseth et al. (4). All samples were mounted on carbon stubs with carbon cement and coated with gold to make them conductive. The investigation was carried out with a JEOL JSM 35 scanning electron microscope (SEM) fitted with a Princeton Gamma Tech PGT-1000 EDS system. The microscope was operated at 25 kV in the secondary electron mode. The analyzing time for the X-ray microanalyses was 60 s.

Samples for X-ray diffractometry were prepared by filtering lung-tissue ash dispersions through Celas silver membranes, which were introduced into a Philips X-ray diffractometer equipped with a rotating sample holder and a graphite crystal monochromator. The mineral phases in the samples were identified from a comparison of the diffraction peaks with those of the Joint Committee on Power Diffraction Standards (5).

Fig. 1. Transmission electron micrograph showing electron-dense deposits of titanium dioxide in human lung tissue. (13,000 $\times$)

For the atomic absorption spectroscopy (AAS) ashed residues of tissue samples from the five lung lobes were dissolved in 5 ml of a 40 % solution of ammonium sulfate in sulfuric acid. Titanium levels

were determined by flameless and flame AAS of the same samples. Copper, iron and zinc were determined by conventional flame AAS. Corresponding analyses were performed on two control lung samples from persons with unknown exposure to titanium pigments. Flameless AAS was performed with a Perkin Elmer model 300 instrument equipped with a HGA-76 graphite furnace, a model 56 pen recorder, and an AS-1 autosampling system. A Perkin Elmer model 403 instrument, equipped with hollow cathode lamps and a Hitachi-Perkin Elmer model 159 pen recorder, was used for the conventional flame atomization. Titanium was determined at the 365.0-nm wavelength, and copper, zinc and iron at the 324.7-nm, 213.9-nm, and 248.3-nm wavelengths, respectively.

Fig. 2. X-ray spectrum from a microanalysis of the inclusions in fig. 1 showing titanium and iron peaks.

RESULTS

During macroscopic and microscopic examinations of the lung samples large amounts of white, birefractive pigments were seen in all parts of the lungs. No obvious fibrotic changes could be observed.

Fig. 3. Scanning electron micrograph of critical-point dried human lung tissue. (320 X)

Fig. 4. Titanium $K\alpha$ mapping of the area depicted in fig. 3. A titanium-containing material is concentrated in a perivascular area.

Fig. 5. X-ray spectrum from a microanalysis of one of the particles depicted in fig. 6 showing titanium peaks $K\alpha$ and $K\beta$ on the right and aluminum $K\alpha$ on the left. The peak at 2.1 keV represents gold from the coating.

A complete case study including a detailed pathological description will be given elsewhere.

Transmission electron microscopy

Electron-dense inclusions appeared either as single particles, or as aggregates (fig. 1), inside and between lung cells. In some cells, localization of the inclusions was revealed in subcellular structures presumed to be phagosomes or residual bodies. EDS analyses showed the presence of titanium and, in some cases, iron (fig. 2). Moreover, selected inclusions were identified by electron diffraction analysis, which in each case gave a diffraction pattern characteristic of rutile.

Scanning electron microscopy

SEM/EDS studies of critical-point dried tissues may reveal the element composition of particles in the region immediately beneath the surface. In this study, the accumulation of titanium-rich materials in perivascular areas of the lung tissue was demonstrated by means of titanium $K\alpha$ X-ray mapping of the specimen surface (fig. 3 and 4). Localization of the material to other lung tissue structures was not possible by this technique.

Fig. 6. Inorganic mineral dust particles collected on a Nuclepore membrane after low temperature ashing of human lung tissue. (4,000 $\times$)

Single mineral particles were identified after the incineration of tissue samples and subsequent extraction of the soluble inorganic compounds. Energy dispersive X-ray microanalysis with both point and mapping techniques revealed the predominance of titanium particles in the lung residue (fig. 5 and 6). The titanium peaks were accompanied by trace amounts of aluminum.

X-ray diffractometry

The diffraction spectrum obtained by X-ray diffractometry on ashed bulk samples revealed the presence of large quantities

Fig. 7. X-ray diffraction pattern of ashed lung tissue residue collected on a silver membrane.

of rutile. Fig. 7 shows the intensities of the 110, 101 and 111 reflections of rutile. Anatase was not identified. Some silicates often present in human lung tissue were also detected, but crystalline silica was not identified.

Atomic absorption spectroscopy

Table 1 presents the levels of titanium, iron, zinc, and copper in the five lung lobes and the two control samples. The values for titanium, which were obtained from flameless AAS, concurred with those of the flame AAS analyses. The increased concentration of dust in the right middle and lower lobes could also be deduced from ash weight determinations. The percentage of ash of dry tissue weight was 6.5 and 6.8 %, respectively, for these lobes, while the corresponding values for the other lobes were 3.1 % (left and right upper lobes) and 4.4 % (left lower lobe). The mean weight determinations of nonoccupationally exposed human lungs gave 2.69 ± 0.45 % ash of dry weight.

DISCUSSION

The deposition and accumulation of titanium pigment dust in the lungs of workers in titanium dioxide factories have previously been studied by incident and polarized light microscopy (2, 6, 7). A histochemical method for the specific dem-

Table 1. Atomic absorption spectroscopic analyses of some elements in lung tissues obtained during the autopsy of a titanium dioxide-exposed individual and in two control specimens. The values (ranges) are presented as the percentage of or as micrograms per gram of ash weight.

Site	Element			
	Titanium (%)	Iron (%)	Zinc ($\mu\text{g/g}$)	Copper ($\mu\text{g/g}$)
Left upper lobe	19.0—22.5	3.8—12.0	1,388—2,042	458—1,700
Left lower lobe	30.7—39.0	2.9—3.6	1,210—1,373	599—752
Right upper lobe	30.8—38.0	5.7—6.6	327—1,673	463—826
Right middle lobe	41.3—49.0	2.1—2.2	1,411—1,696	469—585
Right lower lobe	39.2—47.0	1.9—3.4	1,627—1,639	605—703
Control specimen 1	< 0.2	9.6	2,553	853
Control specimen 2	< 0.2	9.4	1,864	2,210

onstration of titanium oxides in pulmonary dust deposits has been presented (1), but few authors have used the more powerful electron beam instruments for this purpose. However, the TEM/EDS system has proved to be useful for the identification of titanium-containing particles in human (6) and rat (3) lung tissues.

In addition to the TEM/EDS method, the present study reports on the application of a SEM/EDS system for the identification of titanium in bulk human lung samples and for the analysis of the elemental composition of single particles in lung ash residues. An advantage of the latter technique is the simple preparation procedure and the increased sensitivity achieved through removal of the organic materials.

The unequivocal identification of titanium dioxide cannot be achieved by any of the methods mentioned so far. This identification is particularly important with regard to the possible different biological effects of rutile and anatase (9). In the evaluation of the biological significance of exposure to titanium pigments, the pigments involved must be sufficiently specified both when animal experiments are being carried out and when the importance of deposition and accumulation in human lungs are being studied. In this investigation, the crystalline structure of the pigment was determined by X-ray diffractometry of ashed human lung samples. At the single particle level, the identification and localization of titanium dioxide in lung were demonstrated *in situ* by selected area electron diffractometry. By both methods, the presence of rutile was revealed.

The lung titanium levels determined with the AAS analysis in our study were similar to those reported by Schmitz-Moorman *et al.* (7) and Elo *et al.* (2) in men occupationally exposed to titanium pigment dust for 9–15 years, although the patient had been occupationally exposed for only 3 years. The absence of the pulmonary response to the dust deposits reported by some authors (2, 6) may be due to the relatively short time from the initial exposure to death (6 years). We suggest, however, that our observations support the findings of Zitting and Skyttä (9) that rutile is the biological inert crystalline modi-

fication of titanium dioxide. The reported differences in pulmonary response to titanium pigments may therefore be due to the presence of different crystalline modifications of titanium dioxide. The lung titanium levels reported in this case are a thousand times higher than those found in the lungs of nonoccupationally exposed persons by Tipton and Shafer (8). The values for the other elements listed in table 1 are within the ranges reported for nonoccupationally exposed human lungs, even in the case of zinc, which is used in trace amounts (as zinc oxide) in one pigment quality.

Määttä and Arstila (6) stated that adverse effects of titanium pigments may be induced by the coating substances, e.g., quartz, utilized in the manufacture of titanium pigments. In this case no trace of quartz was found in the lung samples. This result is to be expected since the silica involved in pigment production is of amorphous origin. The elevated concentration of aluminum in titanium-exposed lungs reported by Elo *et al.* (2) is certainly due to the use of alumina as another modifying constituent added during pigment manufacturing. The small aluminum peak found in the X-ray spectra (fig. 5) is probably due to this coating material. Only pigments of the rutile type are coated, a fact that may imply physiological properties different from anatase.

The techniques described in this study may also be used for the detection and identification of particulate minerals in lung tissues when a conventional histopathologic examination of patients with occupational diseases gives no indication of the etiologic agents involved or when no occupational exposure can be traced.

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REFERENCES

1. DE VRIES, G. and MEIJER, A. E. F. H. Histochemical method for identification of

- titanium and iron oxides in pulmonary dust deposits. *Histochemie* 15 (1968) 212—218.
2. ELO, R., MÄÄTTÄ, K., UKSILA, E. and ARSTILA, A. U. Pulmonary deposits of titanium dioxide in man. *Arch. path.* 94 (1972) 417—424.
 3. FERIN, J., COLEMAN, J. R., DAVIS, S. and MOREHOUSE, B. Electron microprobe analysis of particle deposited in lungs. *Arch. environ. health* 31 (1976) 113—115.
 4. GYLSETH, B., OPHUS, E. M. and MOWÉ, G. Determination of inorganic fiber density in human lung tissue by scanning electron microscopy after low temperature ashing. *Scand. j. work environ. & health* 5 (1979) 151—157.
 5. JOINT COMMITTEE ON POWDER DIFFRACTION STANDARDS. *Selected powder diffraction data for minerals 1601*. Park Lane, Swarthmore, PA 1977.
 6. MÄÄTTÄ, K. and ARSTILA, A. U. Pulmonary deposits of titanium dioxide in cytologic and lung biopsy specimens. *Lab. invest.* 33 (1975) 342—346.
 7. SCHMITZ-MOORMANN, P., HÖRLEIN, H. and HANEFELD, F. Lungenveränderungen bei Titandioxydstaubexposition. *Beitr. Silikose Forsch.* 80 (1964) 1—17.
 8. TIPTON, I. H. and SHAFER, J. J. Statistical analysis of lung trace element levels. *Arch. environ. health* 8 (1964) 58—67.
 9. ZITTING, A. and SKYTTÄ, E. Biological activity of titanium dioxide pigments. In: INSTITUTE OF OCCUPATIONAL HEALTH, *Proceedings of the Hungarian-Finnish-Italian-Scandinavian symposium on industrial dust problems, Helsinki, Oct. 27, 1977*. Helsinki 1977, pp. 11—13.

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