

# Lung Lesions Induced by Intratracheal Instillation of Vanadium Pentoxide Powder in Rats

Tadao TOYA\*, Kazuo FUKUDA, Mitsutoshi TAKAYA and Heihachiro ARITO

National Institute of Industrial Health, 21-1, Nagao 6-chome, Tama-ku, Kawasaki 214-8585, Japan

Received September 11, 2000 and accepted December 12, 2000

**Abstract:** To clarify acute toxicity and histopathological changes in the lung after exposure to  $V_2O_5$  powder, rats (SD, male,  $n=66$ ) were observed for 4 weeks after an intratracheal administration of  $V_2O_5$  powder (geometric mean diameter  $0.31 \mu\text{m}$ , geometric standard deviation  $\sigma_g=2.19$ ) at three doses (0.88, 3.0, 13.0 mg/kg body weight). The histopathological lung lesions were developed dose-dependently, and characterized by exudative inflammation, injury of alveolar macrophages, and swelling and mucous degeneration in the broncho-bronchiolar epithelia. Growth rate of the  $V_2O_5$  powder-instilled rat was also retarded dose-dependently. The  $V_2O_5$  powder used was composed of not coagulated but well dispersed particles consisting of vanadium pentoxide of more than 99.8% (w/w) with vanadium tetraoxide of less than 0.2%. The  $V_2O_5$  powder was found to be 8 times more soluble in an artificial biological fluid "Gamble's solution" than in a pure water. From the present findings as well as those from the related literature, it was inferred that the histopathological lesions induced by the intratracheally instilled  $V_2O_5$  powder are caused not only by the  $V_2O_5$  particles *per se* but also by vanadium ions dissolved from the particles into the lung fluid.

**Key words:** Vanadium pentoxide, Lung lesions, Solubility, Particle size, Oxidized states, Intratracheal instillation, Rat

## Introduction

Much concern has been raised about adverse health effects on vanadium and its compounds to which workers exposed in various work environments, such as smelting and refining, welding and cutting of vanadium-rich steel alloy, cleaning and repair of oil-fired boilers and catalysis of chemical productions<sup>1,2</sup>. It was known that oil-fired boilers in ships and stations, oil refineries and catalysis-recycling processes released soot and fly ash containing high concentrations of vanadium pentoxide ( $V_2O_5$ )<sup>1,3,4</sup>. An acute phase of adverse health effects by inhalation of vanadium compounds dust was characterized by irritation of nose with bleeding, rhinitis, and eyes with lacrimation, conjunctivitis, and of the upper respiratory tract, sore throat, persistent violent coughs, audible wheezing in the chest, bronchitis, and bronchopneumonia<sup>4-6</sup>. The acute phase, recognized by animal experiments of

vanadium compounds exposed to the respiratory system, was characterized by tracheitis, bronchitis, bronchopneumonia, emphysema, pulmonary edema and hemorrhagic inflammation<sup>4-6</sup>. Several experimental evidence<sup>7-10, 12, 13</sup> implicate that the lung toxicity of metallic fumes and dust is affected not only by degree of exposure but also by size, solubility and oxidized states of those materials.

The present study was intended to clarify acute histopathological effects of intratracheally instilled  $V_2O_5$  powder on the lungs of rat and to examine their correlations with physicochemical properties of the powder as causative factors of the histopathological lung lesions. Gross and histopathological changes in the lungs and growth rates of  $V_2O_5$ -administered rats were examined as toxicity indices together with solubility of the  $V_2O_5$  powder in artificial biological liquid so-called "Gamble's solution" and distilled water, size distribution and oxidized states of the  $V_2O_5$  powder.

\*To whom correspondence should be addressed.

## Materials and Methods

### *Vanadium powder used*

V<sub>2</sub>O<sub>5</sub> powder of a reagent grade used in the present study was purchased from Wako Pure Chemicals Ltd (Japan).

### *Size distribution and oxidized states of vanadium powder used*

Size distribution of V<sub>2</sub>O<sub>5</sub> powder was determined by a previously described method<sup>11)</sup> with an analytical transmission electron microscope (H-8000, Hitachi Co., Japan). In order to determine the particle size of V<sub>2</sub>O<sub>5</sub> powder, many photographs were taken by the transmission electron microscope (TEM) at a magnitude of  $\times 500$ . Images of these TEM negatives were optically enlarged 5 times on the prints on which each particle was measured its size manually using an optical magnifier of 10 times. Therefore, particle size measurements were done totally at  $\times 25,000$ .

The purity of V<sub>2</sub>O<sub>5</sub> powder was examined by a solid-liquid extraction column method<sup>15)</sup>. The powder was dissolved in a warm 1 M H<sub>2</sub>SO<sub>4</sub> solution and then adjusted to pH 4 with 2 M sodium acetate solution. The dissolved sample was applied to a preconditioned anion exchange column for removing V (V) as oxoacid anion. The concentration of V (IV) in the solution from the column was determined with an inductively coupled plasma atomic emission spectrometer (ICP-AES, Jobin-Yvon JY-138 Ultrace, Instruments S.A. Jobin-Yvon Division, France). The concentration of V (V) was calculated on the proportionality of concentrations between V (IV) and total vanadium.

### *Animals*

Male rats of the Sprague-Dawley strain were purchased from Charles River Japan at the age of 4 weeks and housed in wire-meshed cages (5 rats/cage) in animal facility. The animal room was maintained at a temperature of  $24 \pm 1^\circ\text{C}$ , a relative humidity of  $55 \pm 5\%$  and an automatic lighting schedule of a 12-hr light (8:00–20:00)/12-hr dark (20:00–8:00) cycle. The rats were allowed free access to autoclaved food pellets (CE-2, CLEA Japan) and UV-sterilized tap water.

### *Administration of vanadium powder*

The V<sub>2</sub>O<sub>5</sub> powder was suspended and sonicated in a physiological saline at concentrations of 13.0, 3.0 and 0.88 mg V<sub>2</sub>O<sub>5</sub>/kg body weight. Each 8-week-old rat weighing ( $325.4 \pm 16.1$  g) was administered intratracheally with the V<sub>2</sub>O<sub>5</sub> powder-suspended solutions of 0.5 ml at doses of 13.0, 3.0 and 0.88 mg V<sub>2</sub>O<sub>5</sub>/kg body weight. The control group of rats received saline only. The dose levels of V<sub>2</sub>O<sub>5</sub> powder were determined by our preliminary experiment which

demonstrated that the administration of 96 and 48 mg V<sub>2</sub>O<sub>5</sub> powder/kg caused death of all rats tested within 30 minutes after dosing. The method for the intratracheal instillation of V<sub>2</sub>O<sub>5</sub> powder-suspended solution was described previously<sup>12)</sup>.

### *Histopathological preparation and evaluation of the affected lung*

Five rats each of the V<sub>2</sub>O<sub>5</sub> powder-dosed groups were sacrificed in 3 days, 1, 2 and 4 weeks, and three rats each of the control groups were sacrificed in 1 and 4 weeks after the intratracheal instillation. All the rats were prepared for pathological examination by the method described previously<sup>12)</sup>.

Histopathological changes in the lungs of V<sub>2</sub>O<sub>5</sub> powder-administered rats were examined under a microscope for 9 items of lesion types, and each of the lesion types was scored on a scale of increasing severity from 0 to 4 as described previously<sup>12)</sup>.

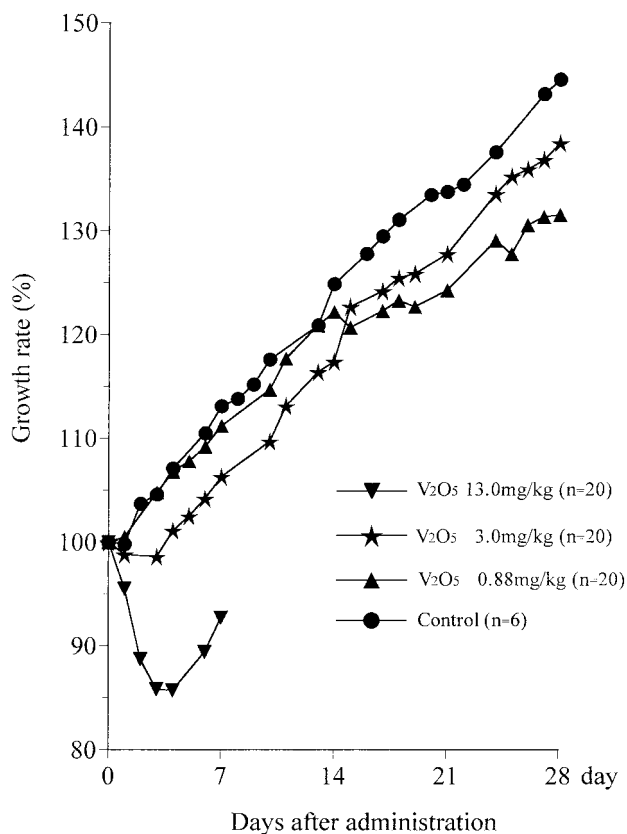
### *Solubility of vanadium powder in distilled water and Gamble's solution*

V<sub>2</sub>O<sub>5</sub> powder of 50 mg was poured into a 15 mL test tube. Then, purified water (obtained by a WF-20 water-purifying system, Yamato, Japan) or Gamble's solution<sup>14)</sup> of 5 mL was added into the test tube. The purified water was prepared by 3 times distillation of distilled water. Gamble's solution was prepared according to the composition reported by Scholze and Conradt<sup>14)</sup>. The V<sub>2</sub>O<sub>5</sub> powder-suspended solutions were kept shaking in a water bath of  $37^\circ\text{C}$  during the time periods of 1, 3, 10, 30, 100, 300 and 1440 minutes. Then, each suspended solution was decanted into a 25 mL disposable syringe (Terumo, Japan) attached with a syringe-driven filter unit (Millipore Millex-HV, hydrophilic PVDF, 25 mm $\phi$ , pore size 0.45  $\mu\text{m}$ , Millipore, USA). A 2 mL of 1M HNO<sub>3</sub> was added with a 0.2 mL portion of the filtered sample solution as a stabilizer. The purified water-based sample solution was added 0.2 mL of Gamble's solution for adjustment of viscosity, and finally diluted to total volume of 20 mL by purified water. Gamble's solution-based sample solution was diluted by purified water to total volume of 20 mL. The vanadium concentrations were measured by inductively coupled plasma atomic emission spectrometer (ICP-AES, Jobin Yvon JY-138 Ultrace, Horiba, Japan) observing wavelength of 309.311 nm.

## Results

### *Growth rate*

Figure 1 shows changes in body weight of 4 groups of rats after a single intratracheal instillation of 13.0, 3.0 and



**Fig. 1.** Growth rates of the rats after single intratracheal instillation of V<sub>2</sub>O<sub>5</sub> powder or physiological saline as the control.

The growth rate was expressed as body weight after the instillation as percentage of that before. The number of rats in each group is indicated in parenthesis.

0.88 mg V<sub>2</sub>O<sub>5</sub> powder/kg body weight and vehicle saline as control. The 13.0 mg V<sub>2</sub>O<sub>5</sub>/kg-dosed group exhibited a significant decrease in body weight for the first 4 days. Then, the survived rats tended to gain their body weight at a rate similar to that of 3.0 mg V<sub>2</sub>O<sub>5</sub>/kg-dosed group. The administration of 3.0 mg V<sub>2</sub>O<sub>5</sub>/kg significantly retarded the growth rate only for the first week. No difference was observed in the growth rate between the control group and the 0.88 mg V<sub>2</sub>O<sub>5</sub>/kg-dosed group.

#### Gross finding

Fourteen out of twenty 13.0 mg/kg-dosed rats (70%) died within the first 4 days. The post-mortem examination indicated exudation of serous or hemorrhagic effusion from nose and foamy liquid from trachea. Some of those rats exhibited retention of serous pleural fluid in the thoracic cavity, marked diffuse coloration with dark red and brown induration of the pulmonary hilus. The six survived rats exhibited atelectasis on the pulmonary hilus, crater-like

induration on the surface of the lobes and petechial hemorrhage surrounding in those lesions. The administration of 3.0 mg/kg caused mild brown induration on day 3 after the intratracheal instillation, but those lesions became slighter in weeks 2 to 4. The administration of 0.88 mg/kg-dosed rats showed slightly focal sporadic dark-red patches over the entire lung surface and a light red coloration of the pulmonary hilus on day 3. Thereafter, there were no observable changes in the lungs.

#### Histopathological examinations of the lungs

Histopathological changes induced by a single intratracheal instillation of 13.0 mg V<sub>2</sub>O<sub>5</sub> powder/kg could be characterized by severe acute exudative inflammations: desquamation and degeneration of swelled broncho-bronchiolar epithelium, hyperplasia of the goblet cell, diffuse hemorrhage, effusions of fibrin and pulmonary edema. It was also remarkable that mobilization and destruction of macrophages and foamy cells, and infiltration of inflammatory cells in the alveolar space.

Table 1 summarizes histopathological changes in the lungs of rats after administration with 3.0 mg V<sub>2</sub>O<sub>5</sub> powder/kg. The 3.0 mg/kg-dosed rats, showed marked desquamation, degeneration, hyperplasia and swelling of the broncho-bronchial epithelium, increased mucous secretion with bronchitis and bronchiolitis. Leakage of plasma, pulmonary edema, hemorrhage and perivascular edema, were also observed together with infiltration of inflammatory cells as well as mobilization and destruction of macrophages and foamy cells (Fig. 2-A, B). These lesions were pronounced for 4 weeks except desquamation in the bronchiole and hemorrhage and effusions of fibrin in the alveolus. The microscopic observations for the lungs of 13.0 mg V<sub>2</sub>O<sub>5</sub> powder/kg-dosed rats revealed that the V<sub>2</sub>O<sub>5</sub> particles were not seen around the bronchioles, alveolar ducts and alveoli where macrophages and other inflammatory cells were abundantly present.

The administration of 0.88 mg V<sub>2</sub>O<sub>5</sub> powder/kg showed similar of histopathological changes as in the 3.0 mg/kg instillation. However, the lesions were milder and disappeared in shorter time than those induced by the 3.0 mg/kg instillation except for swelling and goblet cell metaplasia, mobilization of macrophages and foamy cells (Fig. 2-C). In 4 weeks those lung lesions were recovered except for very slight accumulation of foamy cells in the alveolar space.

#### Solubility, size distribution and oxidized states of vanadium

Figure 3 shows solubility of V<sub>2</sub>O<sub>5</sub> powder in purified water and Gamble's solution. A marked difference in solubility

**Table 1. Lung lesions induced by intratracheal instillation of V<sub>2</sub>O<sub>5</sub> dusts into rats**

Site	Intratracheal instillation Dose/body weight Type of lesion	Single 3.0 mg/kg			
		3 d	1 wk	2 wk	4 wk
Bronchus	Degeneration	2-3	2-3	2	2
	Swelling/Goblet cell metaplasia	4/1	4/3	3/2-3	2-3/1-2
	Desquamation/Regeneration	1-2/2	1-2/2	1/2	0/2
	Reactive hyperplasia	3	3	2-3	2-3
	Granuloma/Fibrosis	0/0	1/0	1/1	1/1
	Inflammation	2	2	1-2	1-2
Bronchiole and Alveolar duct	Degeneration	3	3	3	2
	Swelling/Goblet cell metaplasia	4/1	4/3	4/3	2/1
	Desquamation/Regeneration	1-2/1	1/3	1/3	0/2
	Reactive hyperplasia	3	3	3	2-3
	Granuloma/Fibrosis	0/0	2/0	2/1	1/1
	Fibrosis of Alv. duct	0	0	1	1
	Inflammation	2	2	2	1-2
Alveolus	Mobilization/Destruction of M $\phi$	3-4/3-4	3/3	2-3/2-3	2-3/2-3
	Increase/Destruction of Foamy cells	1/1	3/3	2-3/2-3	2-3/2-3
	Swelling	2	2	1-2	1-2
	Reactive hyperplasia	2	2	1	0
	Granuloma/Fibrosis	0/0	1/1	2/2	1/2
	Thickening/Fibrosis of Alv. wall	2/0	2/0	2/1	1-2/1
	Edema/Hemorrhage	3-4/2-3	3-4/2-3	2-3/1	1-2/0
	Inflammation	3-4	3	2	1
	Fibrin	1-2	1	1	0
Perivascular	Edema	3	3	2	2
	Infiltration	3	2	2	1

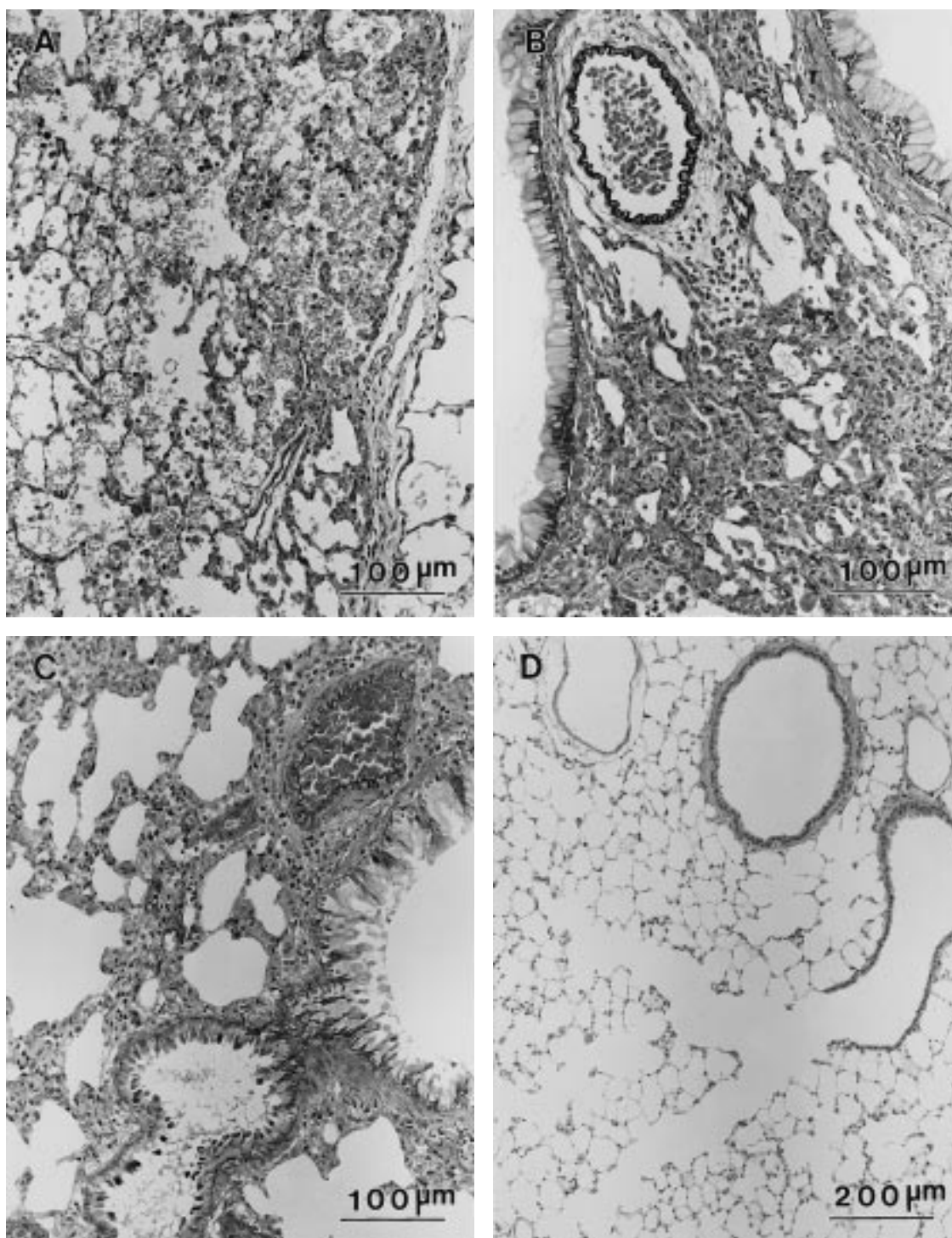
0: Negative, 1: Very slight, 2: Slight, 3: Moderate, 4: Marked.

of V<sub>2</sub>O<sub>5</sub> powder was found between these solutions. The concentration of vanadium ions dissolved in purified water reached a plateau level of 300 ppm (v/v) within the first 5-hr period, whereas the concentration in Gamble's solution increased exponentially to 1900 ppm (w/w) during the first 5-hr period, and thereafter still increased up to 2800 ppm after 24 hr.

The particle size of V<sub>2</sub>O<sub>5</sub> powder used in the present study was measured as 0.31  $\mu$ m in geometric mean diameter with geometric standard deviation  $\sigma_g=2.19$ . It was confirmed by electron microscopic observation that the used powder was composed of the primary particles of less than 1.0  $\mu$ m in diameter and that those particles were not coagulated but well dispersed in water. Chemical analysis of the V<sub>2</sub>O<sub>5</sub> powder showed that the particles contained vanadium (V) pentoxide of greater than 99.8% (w/w) accompanying with less than 0.2% of vanadium (IV) tetraoxide.

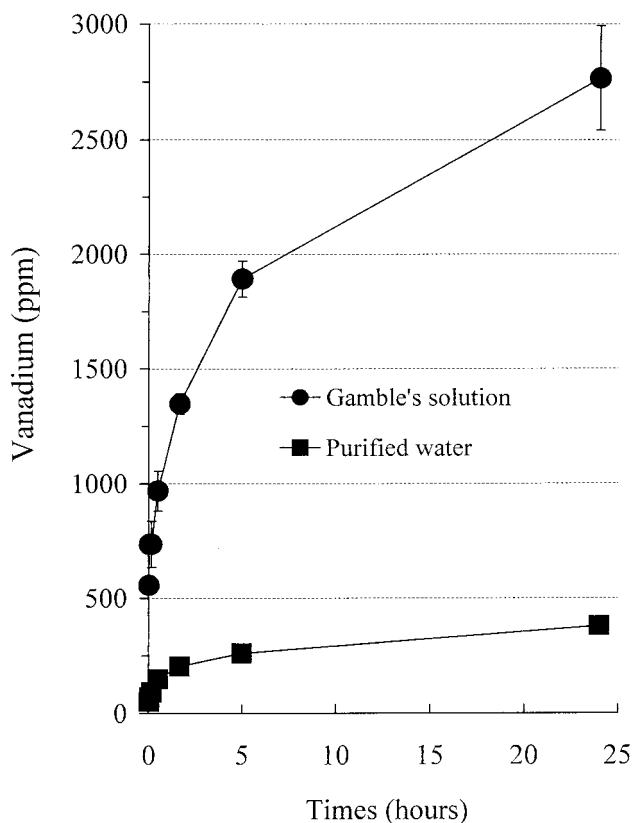
## Discussion

It was found in the present study that intratracheal instilled very fine V<sub>2</sub>O<sub>5</sub> particles in the lungs of rats produced dose-dependent histopathological changes which were characterized by exudative inflammations, injury of alveolar macrophage, and swelling and mucous degeneration of the broncho-bronchiolar epithelium. The growth rates of the V<sub>2</sub>O<sub>5</sub> powder-administered rats were also found to be retarded dose-dependently. It has been reported that affection of inhaled metal particles and fumes on the lungs related not only by the degree of exposure but their size distribution, solubility and oxidized states<sup>7-10, 12, 13</sup>. The findings reported by Zhang *et al.*<sup>8</sup>) and Kyono *et al.*<sup>9</sup>) indicate that higher lung toxicity of ultrafine cobalt powder is related to smaller size and higher solubility of the particles. Serita *et al.*<sup>10</sup>) reported that ultrafine metallic nickel was ten times more soluble in Gamble's solution than in purified water. Toya *et al.*<sup>12</sup>) reported that very fine particles of Ni<sub>2</sub>O<sub>3</sub> were more toxic



**Fig. 2.** A: Focal hemorrhage in the alveoli in the rat lung on Day 4 after instillation of 3.0 mg  $V_2O_5$ /kg b.w. B: Marked goblet cell metaplasia and swelling in the broncho-bronchial epithelia, inflammatory cells and mobilization of macrophages with focal alveolar edema and inflammatory cells in perivascular wall on Day 7 after instillation of 3.0 mg  $V_2O_5$ /kg b.w. C: Marked swelling in the bronchial epithelia and mucous hypersecretion and inflammatory cells in perivascular wall on Day 7 after instillation of 0.88 mg  $V_2O_5$ /kg b.w. D: No histopathological change in the lung on Day 7 after instillation of saline as control rat.

A, B and C were stained by Elastica-Goldoner, and D by HE. Magnification at 165 for A, B and C and at 82.5 for D.



**Fig. 3.** Solubility of  $V_2O_5$  powder in purified water and Gamble's solution, expressed as the concentration of soluble vanadium against a time function of shaking tubes containing  $V_2O_5$ -purified water or  $V_2O_5$ -Gamble's solution.

than ordinary particles of NiO. In the present study, dose-dependent characteristics were found for the  $V_2O_5$  powder in relation to those histopathological lung lesions: It was found by TEM observation and chemical analysis by ICP-AES that the  $V_2O_5$  powder was composed of very fine and well dispersed particles in water, and consisted mostly pure of vanadium pentoxide ( $V_2O_5$ ) particles. It is noteworthy that the solubility of  $V_2O_5$  powder in an artificial biological fluid of Gamble's solution was found to be much higher than that in purified water.

It has been published so far that the vanadium aerosol induces histopathological lesions in the lungs. Roschin<sup>16, 17)</sup> demonstrated nosebleed, hemorrhage of alveolar space, perivascular edema, pulmonary edema and desquamative bronchitis in the rat after inhalation to an aerosol of  $V_2O_5$  at a concentration of 3–5 mg/m<sup>3</sup> for 2 hours in every other day for 3 months and to an aerosol of  $V_2O_5$  at a concentration of 10–30 mg/m<sup>3</sup> for 1 hour in every day for 4 months. The powder sample they used<sup>16, 17)</sup> was obtained by heating vanadium pentoxide in the flame of a Volta arc and by

grinding vanadium pentoxide. Pazynich<sup>18)</sup> demonstrated vascular congestion, focal hemorrhages, and indications of bronchitis in rats after inhalation to  $V_2O_5$  at a concentration 0.027 mg/m<sup>3</sup> for lasting 70 days (24 hours/day, 7 days/week). Kyono *et al.*<sup>19)</sup> observed desquamation of small type epithelium due to edema, diffuse edema in alveolar wall and infiltration of inflammatory cells in alveolar space after short-term inhalation exposure of rats to aerosol of 3.21 mg  $V_2O_5$ /m<sup>3</sup>. Therefore, good coincidence of the histopathological lesions in the present study with those reported so far<sup>16–19)</sup> can be taken to indicate that there is no essential difference in the histopathological lesion between the water-soluble condensed aerosol of  $V_2O_5$  and the  $V_2O_5$  powder used in the present study.

It has been considered that the  $V_2O_5$  particles are not soluble in the lung, because solubility of  $V_2O_5$  in purified water was 0.08%<sup>20)</sup>. In the present study, however, the  $V_2O_5$  powder was found to be much more soluble in an artificial biological fluid (Gamble's solution) than in distilled water. The fine particles was not observed microscopically in the lungs of rats on day 3 after intratracheal instillation of  $V_2O_5$  powder in the present study, while in the previous studies the fine nickel and chromium particles were seen on the same day after intratracheal instillation of those powders<sup>12, 13)</sup>. This difference is due to higher solubility of the  $V_2O_5$  powder in the lung liquid. Thus, the histopathological evidences such as focal edema, hemorrhage, leak of blood plasma and fibrin in the alveolus and perivascular edema found in the present study suggest that the endothelial damage and subsequent leakage of plasma would be caused by toxicity of dissolved vanadium. Therefore, it can be inferred that the lung lesions observed histopathologically are induced not only by the  $V_2O_5$  particles but also by vanadium ions dissolved from the particles in the extra- and intracellular lung fluid.

Dreher *et al.*<sup>21)</sup> demonstrated that the supernatant solution of residual oil fly ash (ROFA) containing Fe, Ni, V, Ca and Mg produced the same extent of acute inflammatory lung injuries as the ROFA suspension *per se* when the two different state of samples were intratracheally-instilled in the rat. Kodavanti *et al.*<sup>22)</sup> demonstrated that pulmonary responses of rats to ROFA differ by virtue of water-soluble metals and that the neutrophilic response to ROFA exposure was positively correlated with the water-leachable content of V. They suggested that soluble transition metals of ROFA play a key role in the pulmonary injury<sup>21, 22)</sup>. Carter *et al.*<sup>23)</sup> also reported that in vitro exposure of human bronchial epithelial cells to vanadium-containing ROFA, but not iron- or nickel-, mimicked the effects of intact ROFA which produced significant amounts of IL-8, IL-6, and TNF, as well as

mRNAs coding for those cytokines. These results reported by Dreher *et al.*<sup>21)</sup>, Kodavanti *et al.*<sup>22)</sup> and Carter *et al.*<sup>23)</sup> seem to be in favor of our inference that the V<sub>2</sub>O<sub>5</sub> powder-induced histopathological lesions are caused not only by the V<sub>2</sub>O<sub>5</sub> particles *per se* but also by the ions.

Waters *et al.*<sup>24)</sup> reported that cell viability after 20-hr in vitro exposure of rabbit alveolar macrophages was reduced by 50% at 13 µg V/ml as V<sub>2</sub>O<sub>5</sub>, 21 µg V/ml as V<sub>2</sub>O<sub>3</sub>, and 33 µg V/ml as VO<sub>2</sub>, and that the cytotoxicity decreased in the order of V<sub>2</sub>O<sub>5</sub>>V<sub>2</sub>O<sub>3</sub>>VO<sub>2</sub> in coincidence with the order of solubility in the medium. Any mechanism underlying the V<sub>2</sub>O<sub>5</sub> powder-induced lung lesions is not clear at present. Zychlinski *et al.*<sup>25)</sup> demonstrated that in the presence of NADPH vanadate is reduced to vanadyl by lung microsomes with concomitant oxidation of NAD(P)H. They also proposed a mechanism underlying vanadium-induced pulmonary toxicity that vanadium (V) undergoes one-electron redox cycling in rat lung biomembranes, and reoxidation of vanadium (IV) initiates lipid peroxidation under an aerobic condition. It is, however, interesting to speculate on a plausible mechanism under which the fine V<sub>2</sub>O<sub>5</sub> powder is taken up by phagocytosis of alveolar macrophages and other cells, leading to production of various cytokines such as inflammatory cytokines<sup>26)</sup>, chemotactic proteins<sup>27)</sup>, interferon<sup>28)</sup> and to reactive oxygen species<sup>29)</sup> and resulting in the acute inflammatory lung injury.

Further study will be needed to clarify soluble chemical species as a causative factor of the histopathological lung lesions induced by administration of V<sub>2</sub>O<sub>5</sub> powder.

## Acknowledgments

The authors express their heartfelt thanks to Drs. Norihiko Kohyama, Yasushi Shinohara, Ayako Takata and Hiroko Kyono for their valuable advice and encouragement in the present study.

## References

- 1) National Research Council (1974) Vanadium. In: Medical and biological effects of environmental pollutants. 167–79, National Academy of Sciences, Washington, D.C.
- 2) Browning E (1969) Toxicology of industrial metals. 2nd ed. 340, Butterworths, London.
- 3) Stokinger HE (1981) The metals. In: Patty's industrial hygiene and toxicology. 3th eds. by Clayton GD, Clayton FE, 2015, A Wiley-interscience publication, New York.
- 4) Lagerkvist B, Nordberg GF, Vouk V (1986) Vanadium. In: Handbook on the toxicology of metals. eds. by Friberg L, Nordberg GF, Vouk VB, 641–51 Elsevier, Amsterdam.
- 5) ACGIH (1996) Documentation of the threshold limit values and biological exposure indices. 1677–80, Cincinnati, Ohio.
- 6) DHHS (1977) (NIOSH) Publication No. 77-222. Criteria for a recommended standard: Occupational exposure to vanadium. 20–80, NIOSH Publications, Washington, D.C.
- 7) Vouk V (1979) General chemistry of metals. In: Handbook on the toxicology of metals. eds. by Friberg L, Nordberg GF, Vouk VB, 24–5, Elsevier, Amsterdam.
- 8) Zhang QW, Kusaka Y, Donaldson K (2000) Comparative pulmonary responses caused by exposure to standard cobalt and ultrafine cobalt. *J Occup Health* **42**, 179–84.
- 9) Kyono H, Kusaka Y, Homma K, Kubota H, Endo-Ichikawa Y (1992) Reversible lung lesions in rats due to short-term exposure to ultrafine cobalt particles. *Ind Health* **30**, 103–18.
- 10) Serita F, Kyono H, Seki Y (1999) Pulmonary clearance and lesions in rats after a single inhalation of ultrafine metallic nickel at dose levels comparable to the threshold limit value. *Ind Health* **37**, 353–63.
- 11) Kohyama N, Tanaka I, Yomiya M, Kudo M, Shinohara Y (1997) Preparation and characteristics of standard reference samples of fibrous minerals for biological experiments. *Ind Health* **35**, 415–32.
- 12) Toya T, Serita F, Sawatari K, Fukuda K (1997) Lung lesions induced by intratracheal instillation of nickel fumes and nickeloxide powder in rats. *Ind Health* **35**, 69–77.
- 13) Toya T, Fukuda K, Kohyama N, Kyono H, Arito H (1999) Hexavalent chromium responsible for lung lesions induced by intratracheal instillation of chromium fumes in rats. *Ind Health* **37**, 36–46.
- 14) Scholze H, Conradt R (1987) An in vitro study of the chemical durability of siliceous fibers. *Ann Occup Hygiene* **31**, 683–92.
- 15) Takaya M (2000) Selective determination method for vanadium (V) and vanadium (IV) controlling the pH of media for a solid-liquid extraction column. *Ind Health* **38**, 91–4.
- 16) Roschin IV (1952) A hygienic description of production vanadium aerosol. *Gig. Sanit* **11**, 49–53 (Rus).
- 17) Roschin IV (1963) Biological effects of rare, dispersed, and other metals and their compounds used in industry-

- vanadium. *Toksikol Redkikh Metal*, 83–95 (Rus).
- 18) Pazy nich VM (1966) Experimental data for hygienic determination of the maximum permissible concentration of vanadium pentoxide in the air. *Gig Sanit* **31**, 8–12 (Rus).
  - 19) Kyono H, Homma K, Serita F, Sawatari K, Suzuki Y, Koshi K, Saegusa J, Fukuda K (1990) Lung lesions induced by the inhalation of metal oxides generated from metals of high melting temperature. *Aerosols* **2**, 1267–70.
  - 20) The merck index (1996) An encyclopedia of chemicals, drugs, and biologicals. 10054, Merck research laboratories, Whitehouse station, NJ.
  - 21) Dreher KL, Jaskot RH, Lehmann JR, Richard JH, Mcgee JK, Ghio AJ, Costa DL (1997) Soluble transition metals mediate residual oil fly ash induced acute lung injury. *J Toxicol Environ Health* **50**, 285–305.
  - 22) Kodavanti UP, Hauser R, Christiani DC, Meng ZH, McGee J, Ledbetter A, Richards J, Costa DL (1998) Pulmonary responses to oil fly ash particles in the rat differ by virtue of their specific soluble metals. *Toxicol Sci* **43**, 204–12.
  - 23) Carter JD, Ghio AJ, Samet JM, Devlin RB (1997) Cytokine production by human airway epithelial cells after exposure to an air pollution particle is metal-dependent. *Toxicol Appl Pharmacol* **146**, 180–8.
  - 24) Waters MD, Gardner DE, Coffin DL (1974) Cytotoxic effects of vanadium on rabbit alveolar macrophages in vitro. *Toxicol Appl Pharmacol* **28**, 253–63.
  - 25) Zychlinski L, Byczkowski JZ, Kulkarni AP (1991) Toxic effects of long-term intratracheal administration of vanadium pentoxide in rats. *Arch Environ Contam Toxicol* **20**, 295–8.
  - 26) Ye J, Ding M, Zhang X, Rojanasakul Y, Nedospasov S, Vallyathan V, Castranova V, Shi X (1999) Induction of TNF alpha in macrophages by vanadate is dependent on activation of transcription factor NF-kappaB and free radical reactions. *Mol Cell Biochem* **198**, 193–200.
  - 27) Pierce LM, Alessandrini F, Godleski JJ, Paulauskis JD (1996) Vanadium-induced chemokine mRNA expression and pulmonary inflammation. *Toxicol Appl Pharmacol* **138**, 1–11.
  - 28) Cohen MD, Mcmanus TP, Yang Z, Qu Q, Schlesinger RB, Zelikoff JT (1996) Vanadium affects macrophage interferon- $\gamma$ -binding and -inducible responses. *Toxicol Appl Pharmacol* **138**, 110–20.
  - 29) Cortizo AM, Bruzzone L, Molinuevo S, Etcheverry SB (2000) A possible role of oxidative stress in the vanadium-induced cytotoxicity in the MC3T3E1 osteoblast and UMR106 osteosarcoma cell lines. *Toxicology* **147**, 89–99.