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Toxicology of High-Fired Beryllium Oxide Inhaled by Rodents

I. Metabolism and Early Effects

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• Several groups of mala and famale rats and hamsters were exposed by inhalation to an nerosol of BeO particles calcined at 1000 C. Initial alveolar depositions ranged from 12μg to 160μg Bo. The alveolar retention half-life for BeO war approximately six months. Only the pulmonary lymph nodes accumulated detectable amounts of translocated BeO. Early alterations were seen in the alveolar macrophages, which were subsequently converted to histiocytic cells that accumulated In subpleural and peribronchiolar granulomatous lesions within eight months after the exposure. The alveolar clearance of a test aerosol, radioactive plutonium dioxide ($^{239}PuO_2$), was decreased to 60% of the normal rate when the radioactive material was given at 1, 30, or 60 days after exposure to BeO. These results demonstrate the important function of the alveolar macrophage in Be-induced granulomatous disease, as well a5 tho rapid impairment of alveolar macrophage function by phagocytized BeO.

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The development of pulmonnry disease in humans has been associated with exposure to compounds containing beryllium during its mining, processing, manufacturing, and utilization stages. Man is esposed to the metal in the common chemical form of beryllium oxide.[1] Recent studies demonstrating the aerosolization of Be from newly ignited lantern mantles would indicate that csposure to Be extends beyond the groups of employees working in industries that utilize the metal.[1]

Due to its greater in vivo solubility, low-fired BeO is considered more toxic in the lung than high-fired BeO.[3,4] Studies with inhaled BeO in rodents have shown progressive development of granulomas, interstitial fibrosis, epithelial metaplasia, and neoplasia in tho lung.[5,6] The more soluble beryllium hydroxide[7] or sulfate[8] compounds exhibit earlier and more profound carcinogenic effects in the lung than BeO. Reports indisatc differences among rodent species in the toxic action of inhaled beryl ore dust, with the rat exhibiting a high incidence of lung tumor, while none is observed in the hamster.[9] It has also been observed that clearance from the lung and translocation of Be to pulmonary lymph nodes differ with the sex of the rodent.[10]

This study describes the deposition, retention, and translocation of inhaled, high-fired BeO in male and female rats and hamsters, and also discusses the important function of the alveolar macrophage in early pulmonary pathologic findings.

METHODS

Male and female Wistar, specific pathogen-free albino rats and Syrian golden hamsters were placed in isolation rooms for thrcr weeks bcforc exposure. All animals were 70 ± 5 days of age at time of exposure. Beryllium oxide was calcined at approximately 1000 C.

Exposure and Aerosol Characterization

The exposure system was designed to provide exposure to BeO via the nose only. The animals were placed in soft-drink bottles that had the bottoms and part of the neck removed. The aerosol chamber was constructed of clear plastic, with exposure ports arranged in seven tiers of 11 ports each. The chamber, aerosol generating equipment, nnd sampling devices were contained within a plastic glove box. The BeO aerosol was generated by a dust feed mechanism, employing R metal cup into which BeO was packed, end it was then passed through a cyclone elutriator for removal of nonrespirable particles. The aerosol was characterized from filter paper samples (aerosol concentration in the chamber), electrostatic precipitator samples (siring of particles from electron micrographs, using an electron microscope and a particle size analyzer), and cascade impactor samples (distribution of particles into eight aerodynamic diameter fractions). An example of the generated BeO aerosol is seen in Fig 1. Exposure periods ranged from 30 to 180 minutes. Concentrations in the chamber ranged from 1μg to 100μg Be/liter. The mass median aerodynnmic diameter for five separate exposures was 1.10μ ± 0.17μ, with a geometric standard deviation of 2.17 ± 0.17 (mean ± SD).

Analytic Determination of Be

Samples containing Be were muffled at 400 C, mixed with nitric acid. and brought to dryness. The ash was digested with 8M HNO_3, 1251 hydrofluoric acid, and 6M hydrochloric acid. The salts were dissolved in acid nntl diluted to 25 ml with distilled water, so that the final dilution contained approximately 2M HNO, and 0.251 HCl. The solution was aspirated into an ntomic absorption spectrophotometer, and absorbancy was rend at 2,319 angstroms. A Be hollow cathode lamp was used in conjunction with a nitrous oxide-acetylene flame. A deuterium background device was used to correct for interference from high salt levels in some samples. Analytical sensitivity was 0.01ppm, or 0.25μg Be per 25-ml sample. Standard curves were determined

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using a standard that contained 1,000 ppm aqueous beryllium nitrate. From 103% to 106% of the Be spiked into lung tissue was recovered by this procedure. Lung tissue samples that were rerun 35 times over several months showed only a 2% variation in Be contents. No background interference was found in Be determinations on tissues or urine. A marked interference occurred with fecal samples that required appropriate corrections in Be determinations.

Biologic Design

The exposures were divided into three groups. In group 1, 35 female and 35 male rats were exposed to a BeO aerosol with an initial alveolar deposition of 138μg to 156μg Be, and 30 female and 30 male hamsters, with an initial alveolar deposition of 16μg to 17μg Be were also exposed. Five animals each of both sexes were killed at 1, 7, 14, 21, 35, 49, or 63 days after exposure for study of Be distribution.

In group 2, 210 male and 210 female rats were exposed to BeO aerosols. These animals were divided equally, receiving three dose levels of beryllium with the following alveolar depositions: 1μg to 2μg, 12μg to 18μg, and 62μg to 104μg. Five rats per group and sex were killed seven days after exposure, and five females per group were killed eight months after exposure for determination of Be distribution and histopathology. The remaining rats, along with 200 nonexposed control rats, are being held for life-span observation.

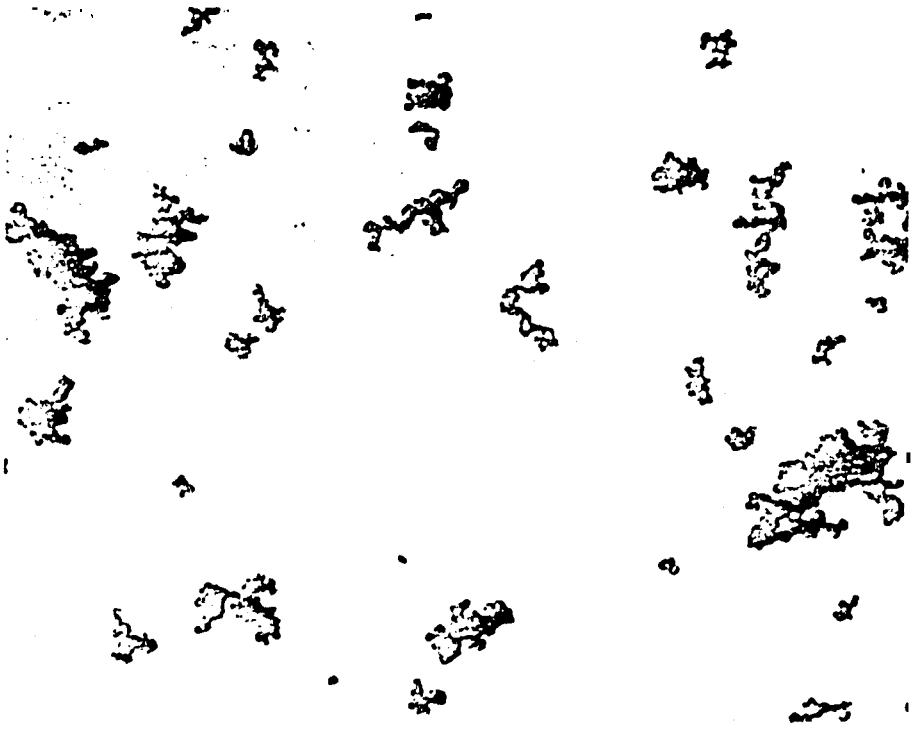
In group 3, 25 female rats were exposed to BeO aerosol with an initial alveolar deposition of 30μg Be. Five rats were killed at 60 days and five were killed at 90 days after exposure for study of Be distribution. Three groups of five rats each were given an additional inhalation exposure to $^{239}PuO_2$ (of count median diameter 0.2μm and 30-minute exposure time) at 1 day, 30 days, or 60 days after exposure to BeO. Three other groups of five rats each, unexposed to BeO, were also exposed to $^{239}PuO_2$. The ^{239}Pu contents of excreta and tissues were analyzed.[11] The exposure system to PuO_2 for rats has been previously described.[12] Five male and five female rats and hamsters from group 1 were placed in individual metabolism cages, immediately following exposure. Excretion were collected from zero to three days after exposure and at weekly intervals up to 63 days after exposure. The rats exposed to $^{239}PuO_2$ aerosol in group 3 were also placed in individual metabolism cages. The excreta were collected in a similar manner up to 30 days after exposure to Pu.

Feces, urine, lung, pulmonary lymph nodes (including thymus, trachea, and main stem bronchi), pulmonary lavage fluid, liver, skeleton, and residual carcass were assayed for Be or ^{239}Pu contents.

The lungs from 15 rats from group 1 and three rats from group 2 were instilled with Karnovsky fixative following tracheal cannulation. They were then fixed in 1% osmium tetroxide, embedded in methacrylate, stained with uranyl acetate and lead citrate, and examined with an electron microscope.

All the other rats in group 1 and those killed in group 2 underwent tracheal cannulation, and the lungs were lavaged with two 10-ml portions of diluent. The combined lavage fluid from each lung was diluted 20:1 and the white blood cell count was determined after the lysing of red blood cells. Cardiac blood was obtained at autopsy by syringe and mixed with ethylenediamine tetraacetic acid. Red and white blood cell counts and white cell differentials of Wright-stained blood smears were determined. The cardiac lobe of the lung from most rats was fixed in 10% neutral buffered formaldehyde solution, embedded in paraffin, and the sections were stained with hematoxylin-eosin for examination under the light microscope. Selected paraffin sections from rats exposed to $^{239}PuO_2$ were coated with nuclear emulsion, and radioautograms were examined after a two-week exposure.

Fig 1.—Beryllium oxide obtained from aerosol chamber by electrostatic precipitation. Grid shadowed with chromium at 22.5° angle (hematoxylin-eosin, ×20,000).



RESULTS

Group 1

The body weights of male rats ranged from a mean of 406 gm one day after exposure to 532 gm 63 days after exposure. Female rat weights ranged from a mean of 310 gm to 370 gm, while male and female hamsters had mean body weights ranging from 108 gm to 130 gm. Body weights and weights of spleen, liver, kidney, and lung of rats exposed to BeO were not noticeably different from controls for all groups. There was an approximate doubling of adrenal weights of both male and female rats and hamsters in group 1 between 21 and 49 days after exposure to BeO. Adrenal weights returned to normal within 63 days after exposure. Red and white blood cell counts in rats and hamsters were not noticeably different from control values.

There were few generalized pulmonary pathologic findings in either rats or hamsters up to 63 days after exposure, except for the alveolar macrophage. Only a few small areas of subpleural or peribronchiolar granulomatous reaction were seen in rodent lungs, these being mild reactions with minimal lymphoid cell involvement.

Within a month after exposure, alveolar macrophages assumed a foamy appearance. Under the electron microscope, we observed an apparent accumulation of eosinophils in alveolar capillaries, as well as a moderate increase in membranous debris in the alveolar air spaces. The most marked alterations were seen in the alveolar macrophage. Beryllium oxide particles were seen in many macrophages, specifically localized within phagosomes or later within phagolysosomes. Within a few weeks, macrophages developed numerous large, clear vacuoles in their cytoplasm. These cells became greatly enlarged, exhibiting a highly vacuolated a p pearance, with cytoplasmic vacuoles containing lysosomal material, BeO particles, membranous debris, and other products of cell degeneration and death (Fig2). In the limited number of sections examined, Beo particles were not seen within type 2 epithelium. The number of pulmonary cells removed by pulmonary lavage niter rats inhaled BeO was not substantially different from controls, although the amount of Be present in lavage samples tended to correlate with the number of cells present.

A total of 483μg and 918μg Be was excreted by female and male rats. respectively. during the 63-day period following exposure; approximately 95% of those mounts was excreted in the feces. The mean total respiratory deposition of Be in rats was 605μg in females and 1,041μg in males. Of these amounts, 22.9% and 15.0%, or 138μg and 156μg Be, were initially deposited in the alveoli of female and males, respectively. A total of 33μg and 37μg Be was deposited in the lungs of female and male hamsters, respectively, and of this, 42% to 51% was deposited in the alveoli (Table 1). From 12% to 21% of the inhaled BeO was cleared from the alveoli of rats within 63 days after exposure, as compared with a clearance rate of 35% to 45% for hamsters. Male rats or hamsters cleared more BeO from the alveoli than did fcmalcs (Tables 1 and 2).

In both sexes, the amount of Be present in pulmonary lymph nodes increased to approximately 1.7% of ini-

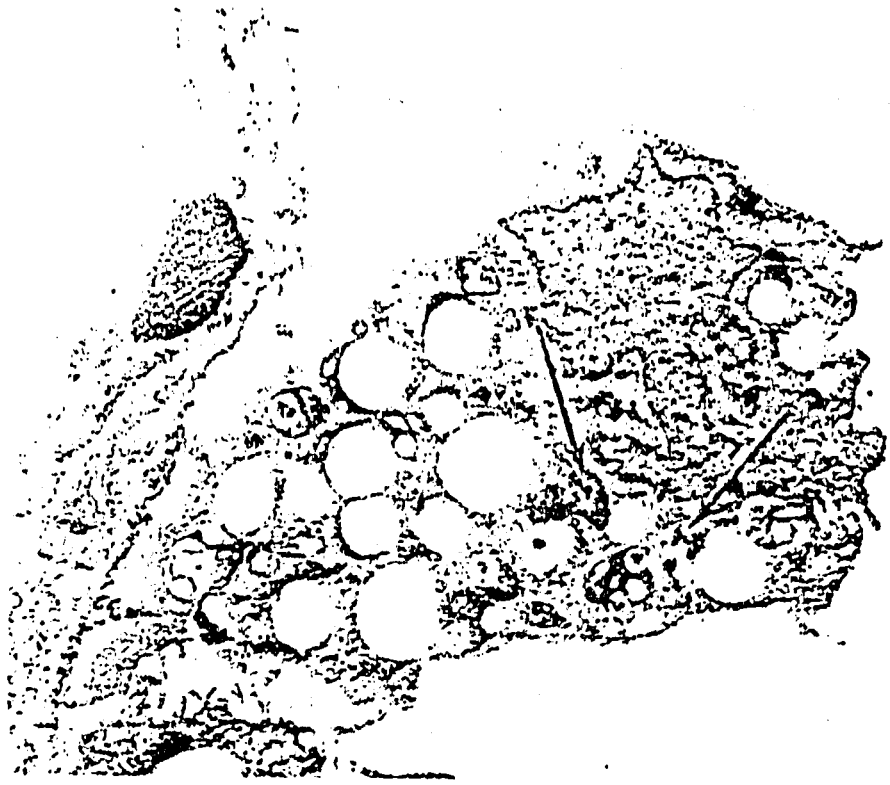


Fig 2.—Alveolar macrophages after inhalation of BeO particles. Top, After 21 days. Bottom. After 49 days. Arrows, Subcellular location of BeO particles in phagosomes (uranyl acetate-lead citrate. X 10,600).

tial alveolar deposition within 63 days after exposure (Table 1). No detectable Be was found in liver, skeleton, or urine seven days after csposurc, or in the feces 21 days nftcr csposurc.

Group 2

Six groups of 70 rats each, equally divided between the sexes, were exposed to BeO ncrosols. Five rats per group were killed seven days nftcr exposure, nnd five female rats were killed approximately eight months aftcr csposurc. The Be contents in tlic lung at seven days were 2.03μg ± 0.7μg, 12.3μg ± 11.3μg, and 104μg ± 49.3μg for males, and 0.9μg ± 0.3μg, 18.2μg ± 15.0μg, and 62.0μg ± 17.2μg for fcmalcs. The amount of Be retained in the lung of female rats eight months after exposure ranged from 24% of initial deposition (a seven-day value) in the alveoli for the intermediate dose group to 36% of deposition for the high-dose group (Table 2). Eight months after exposure, Be content in the lung of the lowest dose group was below detection limits. The biologic half-time for BeO in rat lung was estimated to be approximately six months. The amount of Be in pulmonary lymph nodrs had increased to 4.2% of initial alveolar deposition within eight months aftcr csposurc.

The white blood cell count of pulmonary lavage samples in the highest exposure group was substantially higher than counts in lavage samples from nonexposed rats, although the amount of Be present was similar in samples taken within 14 to 63 days after exposure.

The development of pulmonary pathologic findings was primarily limited to rats that were exposed to tlic highest level of BeO. Moderately developed granulomatous lesions were seen in tho lung. These lesions consisted of alveoli with loosely to moderately packed, dust-laden macrophages that surrounded the peribronchiolar regions or subpleural areas of the lung parenchyma (Fig 3). The macrophages exhibited a foamy appearance and were larger than normal. Lymphocytic infiltration was moderate, and was much less extensive than in acute berylliosis. The granulomatous lesions were widely spaced in the lobes of the lung, and involved only a small portion of the total lung mass. No granulomatous lesions were seen in the nonexposed control. animals. No pulmonary tumors were observed in scheduled autopsied rats, in ten rats dying spontaneously up to one year aftcr exposure to BeO, or in any control rats.

Group 3

One group of female tats was exposed to BeO aerosol; the amount of Be present in the lung at 60 nnd 90 days after exposure was 20μg ± 5.9μg and 12/11: ± 3.2μg, respectively. The test aerosol $^{239}PuO_2$ was given by inhalation at 1, 30, or 60 days after inhalation of BeO, and was then studicd to determine the influcncc of deposition of BeO on the alveolar clearance of ^{239}Pu. The total lung deposition for all groups was approximately 300 nanocurics ^{239}Pu. The alveolar deposition ranged from 20 nanocuries to 38 nanocuries ^{239}Pu (Table 3).

Plutonium particles were distributed only throughout the lung, as determined by radioautograms taken at one or 30 days after exposure to PuO_2. No apparent differences in distribution of PuO_2 in the lung werc seen as a consequence of exposure to BeO, except for an increased number of alpha stars (particles) appearing on the radioautograms of lung from rats that were previously exposed to BeO, as compared to those exposed only to PuO_2.

The amount of ^{239}Pu cleared from the alveoli during the first 30 days aftcr exposure to PuO_2 ranged from 46% to 50% in rats exposed only to PuO_2. Alveolar clearance of ^{239}Pu was deptessed in rats exposed to BeO prior to exposure to PuO_2, with alveolar clearance at 30 days amounting to only 26% to 34% of initial alveolar deposition (Table 3). This amounts to a 40% reduction in alveolar clearance of ^{239}Pu, irrespective of the time at

Fig 3.—Development of early granulomatous lesions in lung of female rat from group 2 (highest dose group) eight months after exposure to BeO. Left, Peribronchiolar region. Right, Subpleural region (hematoxylin-eosin, ×220).

Table 1.—Deposition, Clearance, and Translocation of Inhaled BeO

Parameter	Rat Female	Rat Male	Hamster Female	Hamster Male
Total lung deposition, μg Be	605.0	1,041.0	33.0	37.0
Total lung deposition, all excreta and tissue, %				
Tracheobronchial deposition, all excreta from days 0 to 3	77.0	85.0	49.0	59.0
Alveolar deposition	23.0	15.0	51.0	42.0
Initial alveolar deposition, all excreta* and tissue, %				
Alveolar clearance from day 4 to 63	12.0	21.0	38.0	45.0
Lung at day 63	85.0	77.0	62.0	55.0
Pulmonary lymph nodes at day 63	1.7	1.6	†	†
Other tissue at day 63	0.8	1.2	†	†

* From day 4 to 63.
† Below detection limits.

Table 2.—Retention of Inhaled BeO in the Lung and Translocation to Pulmonary Lymph Nodes*

	Initial Alveolar Deposition, μg Be	Days After Inhalation	Initial Alveolar Deposition, % Female Lung	Female Lymph Nodes	Male Lung	Male Lymph Nodes
Rat	138-156	1	109 ± 19	0.5 ± 0.3	89 ± 35	0.7 ± 0.4
	138-156	7	66 ± 14	0.8 ± 1.0	69 ± 24	1.1 ± 0.6
	138-156	14	82 ± 15	1.6 ± 0.9	56 ± 13	0.9 ± 0.8
	138-156	21	64 ± 19	1.2 ± 0.9	68 ± 21	1.5 ± 0.7
	138-156	35	64 ± 20	1.2 ± 0.6	73 ± 23	0.6 ± 0.5
	138-156	49	66 ± 20	1.7 ± 1.4	...	...
	138-156	63	85 ± 45	1.7 ± 0.7	77 ± 28	1.6 ± 0.9
	18	222	24 ± 11	...	...	...
	62	233	36 ± 14	4.2 ± 2.5	...	...
Hamster	15-17	1	120 ± 47	...	90 ± 27	...
	16-17	7	68 ± 23	...	91 ± 65	...
	16-17	21	30 ± 9.7	...	52 ± 20	...
	16-17	35	51 ± 13	...	46 ± 21	...
	16-17	49	42 ± 16	...	53 ± 19	...
	15-17	63	62 ± 30	...	55 ± 20	...

* Values are expressed as mean ± SD.

which $^{239}PuO_2$ was inhaled after BeO deposition (1, 30, or 60 days).

COMMENT

Early studies with BeO demonstrated its toxicity in the lung.[3,4] Beryllium oxide calcined at 500 C caused severe pathologic damage to the lungs of rats and guinea pigs, while BeO calcined between 1100 C and 1600 C produced only mild granulomatous lesions in the lung.[4]

Up to 68 days after inhalation, the BeO calcined at 1000 C resulted in no atypical epithelial proliferation or granulomatosis in the lungs of the rats and hamsters studied, other than the formation of large, foamy macrophages or histiocytes in the alveoli of animals at the highest exposure levels. Consolidation of histiocytes was not observed. The structure of the alveolar walls was normal. A mild granulomatous reaction was seen in female rats eight months after exposure at the highest dose. Lesions were located primarily in the subpleural and peribronchiolar regions of the lung, and were comprised mostly of histiocytes, lymphocytes, and occasional plasmacytes. Histiocytes contained particulate material, consistent in structure with that of particulates found in the BeO aerosol. Other studies have demonstrated the Be concentration in pulmonary granulomas after inhalation of Be compounds.[11] The lesions found in our rats might be classified as a mild form of group II lesions.[14] Pulmonary lesions attributable to exposure to Be were not found in any other group of rats.

All of the fine structural alterations

Table 3.—Clearance of Inhaled $^{239}PuO_2$ After Prior Deposition of BeO*

Treatment	Excreta, 0-3 Days	Excreta, 4-30 Days	Lung, 30 Days	Total Body, 30 Days	Initial Alveolar Deposition	Alveolar Deposition Cleared in 30 Days, %
	Amount of $^{239}PuO_2$, Nanocuries					
PuO_2 only	290 ± 170	8.5 ± 4.6	11 ± 4.9	12 ± 5.5	20	48 ± 9.6
PuO_2 given 1 day after BeO	310 ± 190	5.9 ± 1.2	18 ± 10	19 ± 11	25	26 ± 8.9†
PuO_2 only	340 ± 200	13 ± 4.9	16 ± 6.4	17 ± 6.6	30	50 ± 8.8
PuO_2 given at 30 days after BeO	270 ± 110	5.9 ± 5.1	18 ± 9.2	19 ± 9.7	29	34 ± 5.7†
PuO_2 only	270 ± 130	15 ± 8.5	16 ± 6.6	17 ± 6.7	32	46 ± 17.0
PuO_2 given at 60 days after BeO	320 ± 68	12 ± 4.5	25 ± 4.6	26 ± 4.8	38	30 ± 8.4

* Values are mean ± SD. Amounts of Be present in the lung at 60 and 90 days after exposure were 20 μg ± 5.9 μg and 12 μg ± 3.2 μg respectively.
† Significantly less than group not given BeO ($P < .05$)

seen in the rats during the first two months after exposure were confined to the alveolar macrophage. After exposure, particles were rapidly phagocytized by macrophages. Clearly identifiable BeO particles were not found in cells other than macrophages during the first 63 days after inhalation. A more thorough search with the electron microscope might have revealed particles in type I alveolar epithelial cells, since inhaled metal oxide particles have been identified within type I cells soon after their inhalation by rats.[15-18]

Evidence of damage to alveolar macrophages became visible shortly after exposure. Cytoplasmic vacuolization and damage to cytoplasmic organelles were seen within two weeks after exposure. The prolonged retention of BeO in the lung of rats, and to a lesser degree, in hamsters, was probably due to macrophage damage caused by the engulfed particles. Deposition of the test aerosol $^{239}PuO_2$ demonstrated an inhibitory effect on the PuO_2 clearance from the lung of essentially the same magnitude, whether the PuO_2 was given at 1, 30, or 60 days after exposure to BeO. Although alveolar clearance was noticeably decreased by BeO at one day, there was no evidence at that time of fine structural damage to macrophages, other than the presence of particles within phagosomes. It seems apparent that phagocytized BeO resulted in decreased mobility of alveolar macrophages or in decreased phagocytosis of PuO_2 particles that were subsequently deposited, which would explain the depressed alveolar clearance of the second aerosol.

A small but not unremarkable amount of Be (1% to 2% of initial alveolar deposition) was translocated to the pulmonary lymph nodes of rats; in female rats, the amount increased to about 4% by the eighth month after exposure. The only species difference observed in the metabolism of inhaled BeO was a more rapid early alveolar clearance in hamsters than in rats. In both species, there was a slightly greater clearance in the male than in the female. No evidence of in vivo solubilization of BeO was seen in either species, as evidenced by the lack of detectable Be in liver, bone, and other extrathoracic tissues.

We conclude that high-fired BeO produced rapid and specific functional and structural damage to the alveolar macrophage, with resultant prolonged retention in the alveoli. Subsequent conversion of BeO-laden alveolar macrophages to histiocytic cells and their loose aggregation in the subpleural or peribronchiolar regions of the lungs produced the mild granulomatosis reaction observed in rats eight months after exposure. High-fired BeO in the lung may be credited more with the impairment of pulmonary clearance of inhaled toxic particulates than with the production of Be disease.

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