State of Art

The Pulmonary Toxicity of Beryllium[1-3]

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Introduction

Even today beryllium disease of the lung is not well understood. This may be due in part to its short history—the first reported case occurred less than 60 yr ago—and in part to the unusual mechanisms of its toxicity.

Many have good reason to be interested in beryllium-induced disease. Industrial workers and those seeking to protect them have often found it a disabling, incurable disease. Epidemiologists have been puzzled by its seemingly erratic prevalence in exposed populations. Industrial hygienists and manufacturers have found difficulty meeting a Permissible Exposure Limit of just 2 $\mu g/m^3$ of air, a level that is lower than that of any other metal. Clinicians were initially confused by the seeming existence of 2 different diseases, an acute one and a chronic one, and by early reports that it was inhaled fluoride and not beryllium that was causing illness at beryllium plants. They were also frustrated by the lack of effective strategies to treat beryllium disease. To immunologists and respiratory biologists, beryllium demonstrated an unusual cell-mediated immunity and has served as a model for other granulomatous lung diseases.

This review summarizes research on the pulmonary effects of beryllium and integrates those results into a model of its pulmonary toxicity that could help to explain some of the puzzles that beryllium disease has posed. The human clinical and epidemiologic literature as well as *in vivo* and *in vitro* experimental work on nonmalignant respiratory effects of beryllium are reviewed. Omitted are considerations of beryllium's extrapulmonary effects and its possible carcinogenicity; these features have been discussed elsewhere (1–4).

Background

The hazards of silica, coal dust, and asbestos have been recognized for many years, and the spread of their pneumoconioses came about with the first Industrial Revolution. In contrast, beryllium disease is a product of the second Industrial Revolution—the twentieth century arrival of the "high" technologies of nuclear power, aerospace, and electronics.

Discovered in France in 1798, beryllium was not produced commercially in the United States until the 1930s (5). The metal's value stems from several useful properties: it has an unusually high melting point, an exceptionally low density, a high modulus of elasticity, a low coefficient of thermal expansion, and a high stiffness-to-weight ratio. Beryllium has good thermal and electrical conductivity. It is relatively transparent to x-rays and has a low neutron absorption cross section. This last property makes it useful as a moderator in nuclear reactors (5).

Beryllium occurs naturally in soils, at an average concentration of 6 ppm, and in coal (5). Coal combustion is the principal source of exposure to beryllium for the public, accounting for more than 90% of the total beryllium emissions into ambient air in the United States (5). But air concentrations are low even in urban areas, and occupational exposures represent the major health hazard to humans.

The beryllium industry initially relied on domestic sources of the ore beryl (a beryllium aluminum silicate) in the form of very large crystals that were handpicked from surface deposits. As demand outstripped this limited source of ore, techniques for processing low-grade ores were developed. A single domestic mine now provides most of the ore consumed in the United States. Ore is also imported from about a dozen countries, the leaders currently being Brazil and the People's Republic of China (6).

The first major use for beryllium was in phosphors for fluorescent lights. Beginning in the late 1930s, numerous cases of disability and death from beryllium poisoning occurred among workers in the fluorescent lamp industry (7). A disease called "Salem sarcoid" (because it occurred around Salem, Massachusetts and resembled sarcoidosis) was shown subsequently by Hardy (7) and others to be caused by inhalation of beryllium. At the same time, cases of acute pneumonitis began to appear in workers at beryllium refineries in Ohio and Pennsylvania (7).

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Starting in 1947, the disease was also observed in residents living near the beryllium refineries (8).

Much of beryllium's early history in the United States is closely tied to the development of nuclear weapons and nuclear power. It was used in early nuclear reactors as a moderator to slow neutrons so that they were more effective in initiating fission of uranium or plutonium. During the early years after World War II, the federal government was the major consumer of beryllium, and the Atomic Energy Commission (AEC) was the agency that contracted for the government's needs. The health and safety staff of the AEC took an active interest in the control of health hazards in the factories of their contractors; they performed air monitoring and made recommendations on work practices, air concentrations, and ventilation systems. In 1949, the AEC set air standards for beryllium that essentially are unchanged today (9). For workplace air, an 8-h time-weighted average maximum permissible level of 2.0 $\mu g/m^3$ was established, along with a peak level of 25 $\mu g/m^3$. The beryllium concentration in air around factories was not to exceed 0.01 $\mu g/m^3$. The AEC demonstrated its intent to enforce these standards by including them in all its contracts for the supply of beryllium (10).

The adoption of adequate standards and hygienic practices to control human exposure to beryllium was delayed by disagreement about the cause of the disease being observed in and around the beryllium plants in the 1940s. In 1943, the United States Public Health Service (USPHS) released an influential study which concluded that beryllium was not itself toxic (11). This position was slowly discredited (although the USPHS did not publish a revised position on beryllium toxicity until 1972 [12]), and in 1949, the AEC set the aforementioned air standards. In 1970, the AEC guidelines were incorporated into the standards of the Occupational Safety and Health Administration (OSHA) (13). In 1976, OSHA considered lowering the 2.0 $\mu g/m^3$ standard to 1.0 $\mu g/m^3$, based largely on evidence of carcinogenicity (13). But no new standard was promulgated, and the issue was tabled indefinitely.

Today, beryllium has many uses (table 1). Pure beryllium metal is found in the aerospace and nuclear industries. But beryllium is more widely used in alloys (typically 1 to 4% in copper) which are used in springs, switches, bearings and gears, and electrical contacts and connectors. Domestic and international beryllium production has increased since the 1940s, albeit erratically, and prices have risen along with this increase (6).

Workers are exposed to dusts and fumes of many different beryllium compounds during extraction and fabrication. Aside from the ore, which is relatively nontoxic, all other commercially important compounds of beryllium exhibit significant pulmonary toxicity. However, a full understanding of the relative toxicities of the different compounds and the influence of particle composition, size, and solubility is lacking. Early exposures that occurred in beryllium refining and in the manufacture of fluorescent lamps were chiefly to beryllium oxide and to various beryllium salts (fluoride, sulfate, and others). Internationally, exposure to these compounds as intermediate products of refining may still be important, but domestically, the number of workers now exposed is small. Less than 10% of the U.S. workers estimated to have exposure to beryllium actually work in primary production (14). Rather, most workers are exposed in industries that fabricate and machine beryllium-containing alloys (14).

Toxicology

Early Studies

The earliest reports on the toxicity of beryllium for laboratory animals were contradictory. Fabroni (15), Weber and Engelhardt (16), and others (17, 18) documented lung damage in experimental animals during the 1930s and early 1940s. But in a USPHS report in 1943, Hyslop and coworkers (11) concluded that:

> ". . . beryllium is of itself not toxic . . . it appears that whatever toxicity has been found to occur with the beryllium salts is due to the toxicity of the acid radical such as the fluoride or oxyfluoride, or to an objectionable condition brought about by the hydrolysis of certain salts, such as the chloride or sulfate."

Although the basis for these conclusions is unclear, one phenomenon that may have misled early investigators is that different beryllium compounds have different degrees of toxicity. This reflects variability in their solubility and thus in the biologic availability of beryllium in the different compounds (rather than the relative toxicity of the nonberyllium moieties).

Confusion about beryllium's toxicity persisted as late as 1951 when the British journal *Lancet*, ignoring the by then numerous reports of human disease from beryllium (8, 19–22), editorialized:

> "Beryllium seems to be the Admirable Crichton of metals. . . . To charge such an admirable metal with having poisonous properties is about as distasteful as accusing a trusted butler of stealing the family plate" (23).

TABLE 1

USES AND PROPERTIES OF SOME IMPORTANT BERYLLIUM (Be) COMPOUNDS*

Compound	Total Be Consumption (%)	Formula	Density (*g/cm³*)	Melting Point (*centigrade*)	Uses
Beryllium (metallic)	33	Be	1.84	1290	Nuclear reactors and weapons, inertial guidance systems, aircraft brakes, x-ray tube windows, turbine rotor blades
Beryl	–	$3BeO{\cdot}Al_2O_3{\cdot}6SiO_2$	2.70	1410	Principal beryllium ore
Bertrandite	–	$4BeO{\cdot}SiO_2{\cdot}H_2O$	2.60	–	Another beryllium ore
Beryllium oxide	5	BeO	3.01	2530	Spark plugs, laser tubes, electrical components, rocket engine liners, ceramic applications, intermediate in Be refining
Beryllium fluoride	†	BeF_2	1.99	‡	Intermediate in Be refining
Beryllium copper alloy (typically 2% beryllium in copper)	50				Springs, bellows, gears, aircraft engines, bearings, welding electrodes, electrical contacts

* From reference 5.

† Produced only as an intermediate.

‡ Indefinite; softens at approximately 800° C.

The clean bill of health for beryllium given by Hyslop and coworkers (11) was not decisively refuted until 1950 when Stokinger and colleagues (24–27) published an extensive series of studies on the toxicity of various beryllium compounds. Much of our knowledge of beryllium's toxicity in experimental animals still comes from these studies. These investigators exposed 11 different animal species to beryllium aerosols that were characterized with respect to both concentration and particle size.

In the largest series (24), animals were exposed to a beryllium sulfate aerosol for 14 to 100 days. They described the acute response as having 2 phases. During the first "highly acute (lethal)" phase, the "more susceptible" animals died rapidly and with little lung damage observable histologically. Survivors of this phase would, upon continued exposure, develop increasingly severe pathologic changes including weight loss, anemia, proteinuria, and hypoxemia leading to death after many weeks of exposure. At sublethal doses the overt signs of toxicity were anorexia, weight loss, and anemia. Most of these signs were absent at the lowest levels of exposure. When the investigators measured Pa_{O_2} in dogs exposed to $BeSO_4$ at a dose equivalent to 40 $\mu g/m^3$ Be, they found that 3 of 5 dogs had values of Pa_{O_2} that were 15 to 29% of those measured preexposure.

The data of Stokinger and colleagues convincingly refuted Hyslop's conclusion that beryllium was not toxic. Also, by citing unpublished studies performed by the AEC, in which rats and guinea pigs were exposed with minimal toxic effects to aerosols of monosodium acid sulfate at sulfate ion concentrations equivalent to those used in his beryllium studies, Stokinger further challenged Hyslop's assertion that sulfate or fluoride ions were responsible for the toxic effects of beryllium salts.

In epidemiologic studies, different forms of beryllium oxide had shown different degress of toxicity, and Stokinger and colleagues designed experiments to explain why (26). They found that the smaller particle size and more soluble grades of BeO were more toxic. There was higher mortality in rats as well as greater weight loss and hypoxemia in dogs exposed to smaller, highly soluble aerosols compared with larger, less soluble particles.

Clearance of Beryllium from The Lung

Beryllium particles are slowly cleared from the lungs, a fact that is probably central to the toxicity of the metal. Sanders and associates (28) measured the deposition, clearance, and retention of inhaled BeO in rats and hamsters. They also administered a radioactive aerosol ($^{239}PuO_2$) after BeO inhalation, and measured the effect of BeO exposure on the rate of clearance of the PuO_2. The results showed considerable variation by species and sex. Females of both species had slower beryllium clearance than did males. Reeves and Vorwald (29), studying retention of beryllium sulfate in rats, reported similar sex differences in the rate of clearance. In both sexes, the clearance halftime exceeded 63 days (29). The experiments with PuO_2 showed that beryllium exposure also compromises the ability of the lungs to clear other particles.

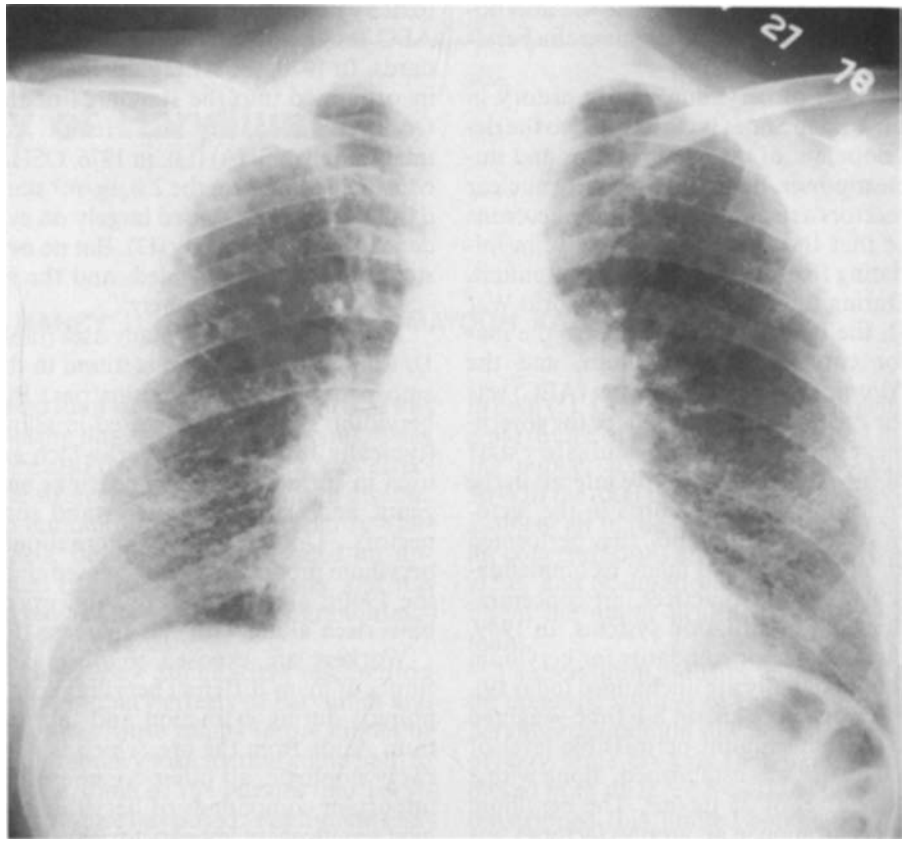

Fig. 1. Chest radiograph from patient with chronic beryllium disease showing irregular opacities in all lung zones, enlarged hilus, and cardiac enlargement. The profusion was classified as s/s, 2/2 in the ILO 1980 classification system.

Beryllium Disease in Humans

Clinical Studies

Acute disease. Acute exposure to beryllium may affect the skin, the mucous membranes, and the respiratory tract. Beryllium is a direct irritant and may cause inflammation and edema of any contacted tissue; it can cause conjunctivitis, periorbital edema, nasopharyngitis, tracheobronchitis, and pneumonitis.

Dermal exposure may cause a primary dermatitis as well as sensitize the skin to subsequent beryllium contact. Dermatitis generally resolves after exposure stops, but ulceration can occur if beryllium particles are retained in the skin.

When the concentration of beryllium particles in air exceeds 100 $\mu g/m^3$, even brief exposures can cause the rapid onset of a chemical pneumonitis (30). Then, symptoms, signs, lung function, and radiographic changes are similar to those found in acute chemical pneumonitis caused by other irritants (31). Common features are dyspnea, cough and sputum, chest pain, tachycardia, crackles, and cyanosis. Diffuse or localized infiltrates are seen on the chest radiograph. Lung volumes are reduced, and there is typically hypoxemia, even at rest.

Treatment of acute beryllium disease includes removal from exposure, bedrest, oxygen therapy and mechanical ventilation, if needed, and corticosteroids. Seventeen percent of patients in one series with acute disease ultimately developed chronic disease, often after a decade or more (31). Acute beryllium disease is now uncommon in the United States because of improved industrial hygiene.

Chronic disease. Chronic beryllium disease is a systemic, granulomatous disease with primary manifestations in the lung. Exposure lasting for months to years is usually required, and the latency between the beginning of exposure and the onset of symptoms ranges from a few months to 25 yr.

Although interstitial granulomata are typically found only in the lungs, noncaseating granulomas may occur also in the skin, liver, spleen, lymph nodes, myocardium, skeletal muscles, kidney, bone, and salivary glands (32). The most common presenting symptom of the disease is dyspnea. This may be followed by weight loss, chest pain, cough, arthralgias, and fatigue. Physical findings include bibasilar crackles, skin lesions, hepatosplenomegaly, clubbing of the nail beds, and lymphadenopathy. In severe cases, cor pulmonale and right heart failure may occur.

Andrews and coworkers (33) studied 41 patients with chronic beryllium disease and reported 3 patterns of impairment of lung function: an obstructive pattern was found in 39% of the patients, a restrictive defect was seen in 20%, and in 36% there were putative changes in the interstitium characterized by normal lung volumes and air-flow rates, but reduced carbon monoxide diffusing capacity.

Chest radiographs from workers with chronic beryllium disease generally show diffuse infiltrates and hilar adenopathy (figure 1). Densities have been described as granular, nodular, linear, and mixed (34, 35). About 40% have hilar lymphadenopathy, which is typically mild, bilateral, and associated with parenchymal changes. Improvement of radiographic changes may occur after corticosteroid therapy.

One of the most difficult clinical problems of chronic beryllium disease is its differential diagnosis from sarcoidosis, a systemic granulomatous disease of unknown etiology (36). A recent report of 5 cases of beryllium disease in which 3 were initially misdiagnosed as sarcoidosis, underscores this problem (37). The Kveim test is reported to be consistently negative in beryllium disease (36), but also has a high false negative rate (20%) in sarcoidosis (38). Thus, a negative result cannot be taken as compelling evidence of beryllium disease when signs and symptoms are consistent with either disease. An additional limitation on clinical use of the Kveim test is that the antigen is not widely available.

The criteria for diagnosis of chronic beryllium disease adopted by the Beryllium Case Registry at the Massachusetts General Hospital require at least 4 of the following 6 features, and must include at least 1 of the first 2 (31): (*1*) epidemiologic evidence of significant beryllium exposure; (*2*) presence of beryllium in lung tissue, lymph nodes, or urine; (*3*) evidence of lower respiratory tract disease and a clinical course consistent with beryllium disease; (*4*) radiologic evidence of interstitial disease consistent with a fibronodular process; (*5*) evidence of a restrictive or obstructive ventilatory defect or diminished carbon monoxide diffusing capacity; (*6*) pathologic changes consistent with beryllium disease on examination of lung tissue and/or lymph nodes.

In the future, improved *in vitro* tests of immune function (discussed below) may enable the differential diagnosis of "occult" beryllium disease (i.e., in patients whose beryllium exposure cannot be identified from history or biopsy), but at present a diagnosis is difficult when based only on clinical, radiographic, and lung function evidence.

Epidemiologic Studies

Morbidity and mortality. Van Ordstrand and coworkers (19), Hardy and Tabershaw (20), and Kress and Crispell (21) first reported beryllium disease in the United States in the early 1940s. By 1948, 400 cases of beryllium disease were known in the United States, and many of the characteristics of the disease established. Machle and colleagues (22) observed that the risk of disease was related to the magnitude of the exposure and the type of beryllium compound. They concluded that the more soluble forms of beryllium seemed to cause the acute disease, whereas the less soluble forms were more likely to be associated with the chronic disease.

Starting in 1947 and continuing into the 1960s, cases of beryllium disease were seen among residents living near beryllium refineries in Ohio and Pennsylvania (7). The disease also developed in family members of workers who brought beryllium home on their work clothes. Air quality surveys were conducted in communities near the plants, and attempts were made to correlate the pattern and frequency of disease with levels of beryllium exposure (8, 39). Although there was a clear dose-response relationship between beryllium concentration in ambient air and the risk of disease in areas close to the Ohio plant, the disease rates outside the plant were unexpectedly high compared with the rates among workers (given that exposure levels outside the plant were far less than those inside the plant).

This observation was one piece of evidence that led Sterner and Eisenbud (1) to propose an immunologic mechanism for beryllium disease in 1951. Citing great variability in the incidence of disease among workers, a toxicity far greater than that of other industrial dusts, a long latency between exposure and disease, a lack of correlation between the amount of beryllium in lung autopsy specimens and the degree of pathologic damage, and the inability (at that time) of toxicologists to recreate the disease in animals, they proposed that there was a mechanism other than "straight chemical intoxication." Two mechanisms were proposed to explain beryllium toxicity: a typical primary irritant reaction, and an immunologic reaction in which beryllium was the specific antigen (1). Others disputed this hypothesis (7), pointing out that the pattern of variable response could also be explained by differences in the level of exposure within the "exposed" group, and a variable latency period.

It is now clear that beryllium can indeed trigger a cell-mediated immune response in addition to being directly cytotoxic. Although Sterner and Eisenbud (1) were largely correct, much of the epidemiologic evidence they cited to support their hypotheses was inadequate. Lacking both the ability to follow an exposed cohort over time and to measure beryllium levels in the air in the plants accurately, serious selection and observation biases limited their ability to estimate the true rates of disease in different exposure groups.

The Beryllium Case Registry (BCR) was established in 1952 by Dr. Harriet Hardy (7). It now contains records of 900 cases of beryllium disease in the United States. Studies from the BCR have addressed the clinical, epidemiologic, radiographic, and differential diagnosis aspects of chronic beryllium disease (7, 33, 40, 41). In 1974, Hasan and Kazemi (40) described 76 new cases added to the BCR since 1966. Approximately half of these resulted from exposure to beryllium after 1949, when the current air standards were generally adopted. Earlier entries had been dominated by workers exposed in the beryllium refining and fluorescent lamp industries, but among the 36 cases exposed after 1949, 81% worked with beryllium metal or beryllium alloys in the aircraft, electronics, and nuclear industries. Typical operations for these workers were grinding and machining. Other

investigators have also reported cases of beryllium disease caused by exposure to beryllium alloys (42, 43). Exposure levels are not known for these cases, but they nevertheless demonstrate that some risk remains even for those involved in relatively "clean" operations using just beryllium metal and its alloys. Industries reclaiming metal scrap may also pose a risk of beryllium disease, and may be especially difficult to control because of the highly variable composition of their raw materials (37, 44).

Subclinical effects of beryllium. The studies just described and others like them established that occupational and ambient air exposure to beryllium caused a characteristic granulomatous lung disease. The risk of this disease among workers exposed during the 1940s and 1950s has been estimated to be in the range of 1 to 10% (45), although these figures must be considered approximate because they are not based on well-defined cohorts. Whatever the precise risks to workers and persons living near plants were, these risks must now be much lower because occupational and environmental controls are more effective. It should not be presumed, however, that current standards are controlling all beryllium health hazards. For example, only a few studies have addressed whether measurable lung dysfunction (e.g., a more rapid decline in forced vital capacity than expected) occurs in exposed persons who do not develop clinical disease.

A cohort study designed to look for nonmalignant respiratory disease among beryllium workers was begun in 1971 at a beryllium refinery in Pennsylvania in operation since 1957 (46). Health examinations were offered to all workers in the plant in 1971 and again in 1974. At the first examination, 214 of 245 workers were seen. Tests of pulmonary function and blood gases and posteroanterior chest radiographs were obtained, and a questionnaire was administered to assess respiratory symptoms. Conditions in this plant in 1971 were poor, with peak air concentrations of beryllium frequently well in excess of the 25 $\mu g/m^3$ peak standard. Four workers were found to have beryllium disease and were removed from exposure. Nine percent of those studied were hypoxemic ($Pa_{O_2} < 80$ mm Hg), 15% had radiographic abnormalities consistent with interstitial disease, and 11% reported symptoms of chronic bronchitis. The investigators concluded that beryllium exposure in this plant had caused not only clinical beryllium disease but also a "reservoir of nonspecific respiratory disease" (46).

TABLE 2

A SUMMARY OF HISTOPATHOLOGIC CHANGES IN BERYLLIUM DISEASE

Phase I.	Nonspecific inflammatory responses. If exposure is severe, dense cellular exudation, emphysema, and obliteration of normal architecture, may resolve to fibrosis. If exposure is mild, may have no permanent effects.
Phase II.	"Foamy" macrophages containing phagosomes with beryllium particles. Peribronchial lymphoid tissue proliferation, lymphocytes visible in intracellular septae and around dust particles.
Phase III.	Granuloma formation: focal accumulations of macrophages, epithelioid cells, and multinucleated giant cells.
Phase IV.	Interstitial fibrosis, hyperplasia of alveolar septae.

A follow-up study of this plant in 1974 found a substantial reduction in air levels of beryllium in the plant. A total of 156 workers were tested, of whom 123 had been seen in 1971 (47). The investigators reported that while there had been no change in mean levels of pulmonary function (FVC, FEV_1, and PEFR), Pa_{O_2} and $AaPo_2$ had improved significantly in those with hypoxemia in 1971. The latter improvements could not be accounted for by decreases in smoking, treatment for beryllium disease, or changes in the place of residence. There was also a suggestion that radiographic abnormalities may have resolved in some workers who had mild interstitial infiltrates in 1971.

Kriebel and colleagues (48, 49) studied workers at a second U.S. beryllium extraction plant. Pulmonary function and blood gas measurements and chest radiographs were performed on 297 white male workers in 1977. Historical industrial hygiene data were used to estimate lifetime beryllium exposure histories for each worker (48). After adjusting for age and smoking effects, a significant association was found between beryllium exposure more than 20 yr prior to the survey and decrements in FVC and FEV_1 (49). Beryllium exposure in the 10 yr prior to survey was associated with increased $AaPo_2$. These associations, which occurred in workers with no radiographic abnormalities, predicted small but significant lung function changes caused by long-term exposure. For example, a cumulative exposure to beryllium of 100 $\mu g/m^3$ – yr occurring more than 20 yr prior to the health survey was associated with a mean loss of 220 ml of FVC, after adjusting for the effects of age and smoking (49).

The studies of Kanarek and coworkers (46), Sprince and associates (47), and Kriebel and colleagues (49) taken together suggest that elevated exposure to inhaled beryllium causes early, mild lung disease characterized by hypoxemia and mild interstitial infiltrates. Longer term exposure may lead to fibrosis and restrictive defects. It should be stressed that these effects are seen in workers who are free of clinically defined chronic beryllium disease, as described above.

Mechanisms of Disease

Lung Function

We have seen that the earliest functional change reported in humans (46) and in dogs (26) with chronic beryllium disease is an increased $AaPo_2$ or mild hypoxemia. Later changes, observed in clinical studies of patients with beryllium disease, include a variety of pulmonary function deficits. The occurrence of both restrictive and obstructive changes can plausibly be explained by different anatomic patterns of granulomatous lesions—interstitial granulomas producing restrictive deficits and lesions in or around airways creating an obstructive deficit. Despite this mixture of patterns, restrictive changes do appear to predominate (33, 35). Clinical consequences of late progressive beryllium disease are typical of many fibrotic lung diseases: cor pulmonale, pulmonary hypertension.

Histopathology

Many studies in animals have described the histopathologic aspects of beryllium disease (28, 50–57) (table 2). Animals typically show an early inflammatory response with histopathologic features indistinguishable from those caused by other strong irritants. If the doses are high enough, this phase may be rapidly fatal. If not fatal, there may be either resolution or progression to the next phase, probably depending again on the dose. Species differences exist, and the work of Barna and coworkers (52, 53) on guinea pigs (described below) suggests

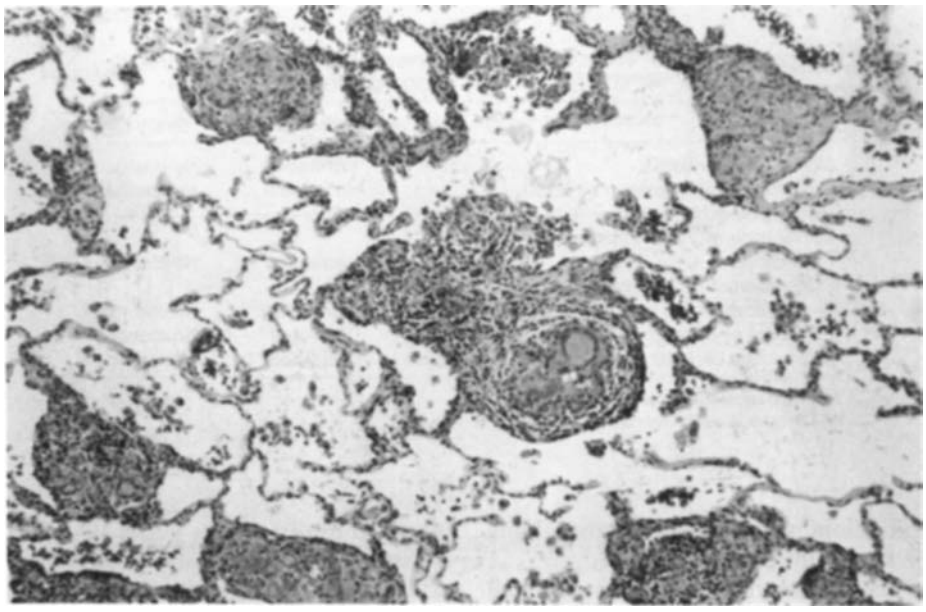

Fig. 2. Granulomas and mild interstitial infiltration in lung biopsy of a patient with chronic beryllium disease.

that these differences may be related in part to the nature of the immune responses to beryllium. The second and third phases in the response to beryllium also include an immune component. Granuloma formation, the hallmark of the chronic disease in humans, develops in a number of animal species (the rat and guinea pig are the best studied) (28, 51–54, 56, 57) after either a single dose or prolonged administration of a beryllium compound (figure 2). The final phase, like the first, is similar to that seen with many other pulmonary toxins; it is a progressive, often fatal, interstitial fibrosis.

Cellular Responses

The macrophage: uptake and clearance. Alterations in the appearance of alveolar macrophages are among the first changes to be detected after experimental exposure to beryllium. Sanders and associates (28) described the macrophage early after beryllium exposure as being "foamy" in appearance because it contained many vacuoles. It is likely that the persistence of beryllium inside macrophages for long periods explains the slow clearance of inhaled beryllium particles from the lungs (28, 29).

Hart and Pittman (58) showed that beryllium uptake was reduced in the presence of inhibitors of glycolysis, respiration, and microfilament formation (cytochalasin B). In a later study, Hart and colleagues (55) examined macrophages in rats that were instilled with BeO and then followed for as long as 21 days. Total numbers of macrophages recovered from lung lavage fluid dropped by two-thirds 2 days after exposure, but returned to normal within 21 days. Macrophage phagocytic ability *in vivo* was impaired during the first week, but increased well beyond normal levels by Day 21.

Camner and colleagues (59, 60) studied the fate of inhaled Teflon spheres coated with beryllium and other metals in intact rabbits as well as in rabbit alveolar macrophages studied *in vitro*. They found that beryllium-coated 5-μm spheres were taken up less effectively by macrophages than were similar spheres coated with silver because the beryllium layer was toxic to the macrophages.

Kang and colleagues (61) exposed rabbit alveolar macrophages to BeO and $BeSO_4$ *in vitro* and measured the production and release of enzymes. Release of lysosomal hydrolases was increased by both beryllium compounds, although higher doses of BeO were required. Both compounds were toxic to the macrophages, and viability was only about 45% (Hart and Pittman [58] used lower concentrations of beryllium and had viabilities of greater than 90%). Kang and colleagues (61) reported that lysosomal hydrolases were released without an increase in intracellular levels, implying the release of stored enzymes rather than an increased production.

In summary, alveolar macrophages take up particulate beryllium compounds by phagocytosis, are damaged to some extent in that process, and release lysosomal enzymes. The beryllium deposited in alveoli is not cleared very readily. Macrophage phagocytic activity may be decreased initially and then later stimulated by beryllium exposure. These findings suggest that macrophages sequester beryllium in the lung; in the process they may become more active, with potentially damaging effects on lung architecture. Their role in immune response to beryllium is discussed below.

Immune responses. The immunology of beryllium disease is perhaps its most interesting aspect and the one that is currently most heavily studied. Beryllium's ability to trigger a cell-mediated immune response in the lung (62–64) sets it apart from most other toxic dusts.

As early as 1951 (65), it was shown that a skin patch test could identify some workers as "allergic" to beryllium. The usefulness of this test has been limited because sensitization and the presence of pulmonary disease are not well correlated, and because the test itself can induce beryllium sensitization and thereby provoke symptoms in previously healthy workers (66).

More recently, *in vitro* tests for detecting beryllium sensitization have been developed (65–69). These tests identify sensitized T-cells recovered from peripheral blood or from bronchoalveolar lavage (BAL) fluid. The T-cells are cultured in the presence of beryllium, and then either macrophage migration inhibition or enhanced lymphocyte transformation is assessed (70). Peripheral blood lymphocytes generally test positive when obtained from patients with beryllium disease, and negative in healthy unexposed control subjects. However, there is considerable variability in the ability to correctly identify patients with beryllium disease among laboratories. These patients are often receiving corticosteroid therapy, and this may inhibit the lymphocyte response. The percentage of patients with beryllium disease who are not sensitive to beryllium in such tests ranges from zero (71) to 30% (67). Limited studies of lymphocytes recovered during BAL suggest that the effect of beryllium on lymphocyte function may be more pronounced in the lungs than in peripheral blood (62–64, 72, 73). Epstein and colleagues (72) first reported on lymphocyte proliferation assays of BAL lymphocytes from a worker with beryllium disease. They found that a higher proportion of BAL lymphocytes than of peripheral lymphocytes were sensitive to beryllium, and that the BAL lymphocytes transformed more rapidly in the presence of

beryllium salts than did peripheral lymphocytes. It has also been observed that the ratio of helper-to-suppressor T-cells in BAL fluid is increased in beryllium disease (63). It is not yet clear how to interpret positive lymphocyte tests in persons exposed to beryllium (66). Is lymphocyte transformation a marker for past exposure or for sensitization to beryllium? If it is the latter, does the test identify persons who are genetically predisposed to this sensitization, or persons who have become sensitized from prior beryllium exposure? It would seem prudent to remove persons found to be sensitive to beryllium from further exposure, but we lack evidence that they are in fact at higher risk of developing beryllium disease than anyone else. Prospective studies of exposed populations who are initially free of disease are needed to distinguish among these possibilities.

Rom and colleagues (74) studied a cohort of 82 beryllium workers and examined the relationships between peripheral blood lymphocyte transformation, beryllium exposure, and lung function over a 3-yr period. They found that lymphocyte transformation was correlated with exposure, but not with the presence of disease, and was reversible upon reduction of exposure. More studies with longer follow-up and larger cohorts are needed to clarify the significance of these *in vitro* tests.

The role of the alveolar macrophage in the immune response to beryllium was investigated by Hanifin and colleagues (69). Alveolar macrophages were exposed to BeO *in vitro* and then removed from the beryllium suspension and rinsed thoroughly. When these macrophages were introduced into a culture of lymphocytes from subjects with chronic beryllium disease, lymphocyte blast transformation was observed. Macrophages incubated with saline did not transform lymphocytes in patients with beryllium disease. These experiments suggest that lung macrophages may present the "beryllium antigen" to lymphocytes, although this phenomenon has not yet been shown *in vivo*. Hanifin and colleagues (69) also reported that circulating monocytes recovered from subjects with beryllium disease and cultured *in vitro* developed into mature macrophages more rapidly than did monocytes from control subjects. This may be because newly isolated monocytes from subjects with beryllium hypersensitivity were already larger and had more granules. They concluded that the "hyperactive monocyte" may lead to the epithelioid macrophage and granuloma of beryllium disease (69).

TABLE 3

A COMPARISON OF THE EFFECTS OF BERYLLIUM OXIDE IN TWO STRAINS OF GUINEA PIG 6 WEEKS AFTER ENDOTRACHEAL INSTILLATION*

	Strain 2	Strain 13
Skin reaction to $BeSO_4$	Increased†	No change
Lymphocyte transformation by $BeSO_4$	Increased	No change
Histopathologic lung disease (0 to 4+)	2+ or 3+	0
Macrophage migration inhibition by lymphocytes	Increased	No change
Proliferation of normal fibroblasts by lymphocytes	Decreased	No change
Proportion of T-cells in BAL lymphocytes	Increased	No change
Macrophage bacteriacidal activity	Increased	Decreased

* From references 51 to 53.
† Relative to control animals.

Because beryllium ions are too small to be antigenic per se, it has been assumed that they must bind to a protein to form a hapten-carrier complex. Jones and Amos (75) concluded that nonsensitized lymph node cells could produce a beryllium antigen upon exposure *in vitro* to a colloidal beryllium compound. They demonstrated the production of the antigen by injecting lymph node cells (after sequential beryllium exposure and washing) into guinea pigs, and then showed that the animals had become sensitized to subsequent beryllium exposure by the dermal route. This sensitization was not produced simply by injection of the colloidal beryllium compound or by a similar amount of beryllium bound to erythrocytes. Because the sensitization could be produced by lymphocytes from strains of guinea pigs that were genetically incapable of being sensitized directly, these investigators suggested the production of an immunogen, possibly on the lymphocyte surface.

Barna and colleagues (51–53) developed a useful animal model for studying the immunologic aspects of beryllium disease. They first showed that outbred Hartley and inbred Strain 2 guinea pigs developed granulomatous beryllium disease after endotracheal injection of beryllium salts. These strains also showed *in vitro* lymphocyte transformation to beryllium salts. Oral or intravenous administration of beryllium sulfate made the guinea pigs more tolerant to subsequent endotracheal exposure. Another inbred strain (strain 13) was found to be resistant to the granulomatous disease; the F1 hybrid (2 × 13) developed a milder disease than did Strain 2 (table 3). The investigators suggest that variation in the susceptibility to beryllium in the 2 strains is related to differences in the Ia region of the major histocompatibility complex; expression of Ia may be an integral part of the response to beryllium. Additionally, Strain 13 demonstrated reduced alveolar macrophage activity after BeO exposure. In contrast, Strain 2 was immunologically responsive, developed granulomatous disease, and responded to BeO with increased macrophage activity.

Although increased levels of serum immunoglobulins have sometimes been reported in humans with chronic beryllium disease (66, 76, 77), the findings were inconsistent. There is currently no convincing evidence that a humoral immune response plays an important role in beryllium disease.

The cutaneous hypersensitivity reaction to beryllium is another intriguing aspect of beryllium immunology. Intradermal or endotracheal injection with beryllium causes a delayed hypersensitivity-type skin reaction in guinea pigs (52, 78). Induction of skin hypersensitivity can confer resistance to pulmonary disease after beryllium inhalation in guinea pigs (78). The ability of guinea pigs to become skin-sensitized appears to be a heritable trait (79, 80), and genetic studies suggest it is not sex-linked (79). It is difficult to know how to interpret the results of these animal studies. They do demonstrate that it is important to distinguish among beryllium's various peripheral and pulmonary immune responses, but the meaning of each component is not known.

In summary, the following evidence supports the existence of cell-mediated immune responses to beryllium: (*1*) lung and lymph node histologic features similar to those caused by other cell-mediated reactions (56), (*2*) the cutaneous sensitivity reaction (65), (*3*) blast transformation and migration inhibitory factor

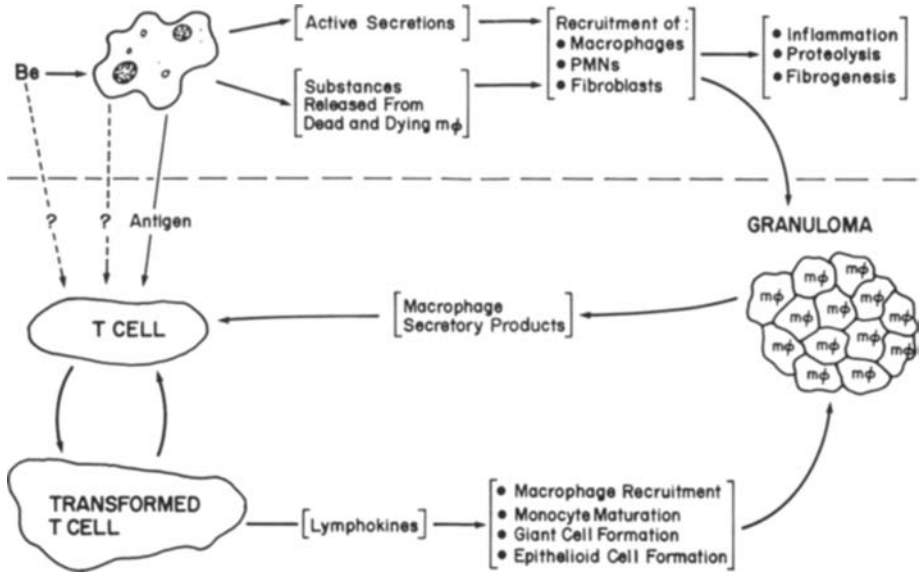

Fig. 3. A model of the pulmonary toxicity of beryllium: the acute inflammatory mechanism (*above dashed line*) and the T-cell-mediated immune response leading to granuloma development (*below dashed line*).

(MIF) production from lymphocytes (67), (*4*) selective stimulation of T-lymphocytes (81), and (*5*) skin sensitivity in guinea pigs that is transferred by lymphocytes but not by serum (66).

A Model of the Cellular Mechanisms of Beryllium Toxicity

Some of the confusion that has surrounded beryllium disease probably stems from the existence of two different but interrelated disease processes. Sterner and Eisenbud (1) characterized these processes as a "regular, primarily irritant, chemical intoxication, and a modified immunologic response in which beryllium is the specific antigen." The interplay of these 2 processes, regulated by dose, dose-rate, elapsed time, and host factors (including genetics and immune status) probably determines the characteristics of the disease in a given individual. Since this concept was proposed in 1951, experimental work on beryllium and an improved understanding of underlying immune mechanisms have confirmed and embellished the original hypothesis; figure 3 summarizes some aspects of this model.

The Inflammatory Response

Beryllium is toxic to cells, and acute beryllium disease in humans is probably caused by this cytotoxicity and by ensuing inflammatory responses. But cytotoxicity undoubtedly contributes to the chronic disease as well. The mild reversible effects reported by Sprince and colleagues (47) may be inflammatory responses that lead to hypoxemia and radiographic changes. Frequently the inflammatory and immunologic components cannot be separated; the inflammatory process may continue even during the development of granulomas in many laboratory animals.

The inflammatory response to beryllium probably has the following main steps. Inhaled beryllium particles bind to the plasma membrane of alveolar macrophages and are then ingested by them. Macrophage function is severely compromised—some are killed and lysed, others are damaged. The release of lysosomal enzymes, chemotactic factors, and mediators stimulates recruitment of additional macrophages as well as polymorphonuclear leukocytes (the latter may be less common than in the response to other inflammatory agents). Inflammatory mediators released by macrophages and neutrophils can also increase capillary permeability, resulting in pulmonary edema. Acute disease in humans often reverses completely with little or no residual scarring, suggesting that fibrosis is not an essential consequence of the inflammatory response to beryllium.

The Immune Response

Comparable cytotoxic changes can also be elicited by other particles such as alpha-quartz. However, beryllium is unusual in that it also initiates cell-mediated immune responses. Beryllium may function as a hapten, binding with some large carrier molecule (for example, a cell-surface or serum protein) to form an antigen. In the lung, an important mechanism may be that beryllium particles are taken up by macrophages that create an antigen (of unknown form), which in turn is presented to lymphocytes and other macrophages. Once a population of sensitized T-cells is created, these cells transform and actively regulate subsequent events via the secretion of lymphokines. Transformed helper T-cells release, in response to subsequent exposure to beryllium antigen, a macrophage migration inhibition factor, a monocyte maturation factor, and probably other lymphokines as well. By inference from other granulomatous diseases, it seems reasonable to assume that interleukin-2, for example, probably is also released by helper T-cells to stimulate the differentiation of additional effector T-cells. These mediators initiate and perpetuate the process of granuloma formation in ways that are probably similar to other granulomatous diseases.

Research Priorities

Epidemiology

The hygienic standards for beryllium that have stood for nearly 4 decades have undoubtedly prevented many cases of beryllium disease. Whether the standards are adequate to prevent all disease is less certain. If, for example, workers could become sensitized by brief, very high exposures, then an 8-h, time-weighted average air concentration is not an appropriate measure on which to base a standard. There has always been a peak standard at 25 $\mu g/m^3$ (currently defined by OSHA as the level not to be exceeded for more than 30 min). Unfortunately, enforcement of a peak standard is very difficult.

In vitro lymphocyte transformation tests are now available. However, before they can be used in diagnosis and screening, longitudinal epidemiologic studies must be performed that follow workers with varying exposures to beryllium and varying status on these tests. Is the subject with a positive test at higher risk of disease from continued exposure (either because of high previous exposure or because of some genetic predisposition) or is he or she simply an individual who has been more exposed to beryllium in the past? Is lymphocyte transformation a measure of subsequent risk or past exposure?

Too little is known about the existence of subclinical effects of beryllium. Sprince and colleagues (47) reported the

existence of reversible effects. If this finding can be replicated, it suggests that disease can be prevented through early detection of these reversible effects, followed by reduction of exposure. However, the fact that some effects are reversible does not preclude other progressive, irreversible subclinical effects. Studies are needed to examine whether workers exposed to beryllium for a long time experience either elevated rates of loss of pulmonary function or increased symptoms of respiratory illness.

Toxicology

Mechanisms of beryllium toxicity also require further study. Emphasis should be placed on the key cells—the alveolar macrophage and the helper T-cell. Important research topics include: the chemical form of the beryllium antigen, the specific steps in its production, the role that macrophages play in this process, the conditions of beryllium dose and bioavailability under which the antigen is produced, the importance of genetic variability in the production of a sensitized population of T-cells, and the importance of this variability in determining the severity of disease produced by beryllium.

Such research may help guide epidemiologic studies and standard setting. For example, delineating the importance of dose-rate versus total cumulative dose is difficult from epidemiologic data, yet it is crucial to the setting of adequate hygienic standards. It is also important to know whether brief, high exposures can sensitize individuals; then subsequent low exposures would be more potent for such a sensitized person than for an unsensitized person.

The relative toxicities of different chemical and physical forms of beryllium is another area where toxicology can make essential contributions. Today, most workers exposed to beryllium in the United States work with alloys containing just a few percent beryllium. However, the toxicity of these alloys is largely unknown. Because most users of beryllium alloys are small plants, it is unlikely that a large cohort of workers with this exposure could be assembled for prospective studies. The challenge for toxicologists is to compare the relative toxicities of these beryllium alloys with the better-studied beryllium oxide, hydroxide, and salts. The results can guide the development of standards for the alloys.

Immunology

Beryllium lung disease can also serve as a useful model of a cell-mediated immune response in the lung, and thus help our understanding of the pathogenesis of other granulomatous diseases. Beryllium triggers a granulomatous response very similar to that caused by the unknown agent responsible for sarcoidosis. Research aimed at both early detection or treatment of beryllium disease will be useful to sarcoidosis research. For example, it is important for progress on both diseases to understand the relationship of T-cells recovered from the lungs to those circulating in the periphery. That knowledge might lead to more sensitive and specific peripheral blood lymphocyte transformation tests. Such tests would be preferable for screening to those using lymphocytes recovered from BAL, since BAL involves both expense and discomfort.

Prevention

Worldwide, beryllium continues to be an important but preventable cause of lung disease. With increasingly diverse uses for this toxic metal, large numbers of workers are exposed. The current hygienic standards are strict, and usually expensive engineering controls and persistent monitoring are necessary. As a result it is likely that in many smaller firms in the developed world, and in most establishments in developing countries, these standards are often not met. Consideration must be given to substitution of less toxic substances when possible; if not feasible, new approaches to controlling hazards, careful monitoring of exposure levels, and improved early detection of disease should be implemented.

References

1. Sterner JH, Eisenbud M. Epidemiology of beryllium intoxication. Arch Ind Hyg Occup Med 1951; 4:123–51.
2. Groth DH. Carcinogenicity of beryllium: review of the literature. Environ Res 1980; 21:56–62.
3. Mancuso TF. Relation of duration of employment and prior respiratory illness to respiratory cancer among beryllium workers. Environ Res 1970; 3:251–75.
4. Infante PF, Wagoner JK, Sprince NL. Mortality patterns from lung cancer and nonneoplastic respiratory disease among white males in the Beryllium Case Registry. Environ Res 1980; 21:35–43.
5. Drury JS, Shriner CR, Lewis EB, Towill LE, Hammons AS. Reviews of the environmental effects of pollutants: VI. Beryllium. Cincinnati: USEPA, Health Effects Research Laboratory, 1978 (NTIS no. EPA-600/1-78-028).
6. Bureau of Mines. Minerals yearbook. Washington D.C.: U.S. Govt. Printing Office, 1985.
7. Hardy H. Beryllium disease: a clinical perspective. Environ Res 1980; 21:1–9.
8. Eisenbud M, Wanta RC, Dustan C, Steadman LT, Harris WB, Wolf BS. Non-occupational beryllosis. J Ind Hyg Toxicol 1949; 31:282–94.
9. Eisenbud M. Origins of the standards for control of beryllium disease (1947–1949). Environ Res 1982; 27:79–88.
10. Donaldson H. Comparison of personal sample collection methods with the AEC sample collection method in a controlled beryllium production facility. 1976. Unpublished report of Environmental Investigations Branch, DSHEFS, NIOSH, USDHHS, Cincinnati.
11. Hyslop F, Palmes ED, Alford WC, Monaco AR, Fairhall LT. The toxicology of beryllium. Washington D.C.: U.S. Public Health Service, 1943 (NIH Bulletin no. 181).
12. Tabershaw IR, ed. The toxicology of beryllium. Washington D.C.: U.S. Public Health Service, 1972 (PHS publication no. 2173).
13. U.S. National Institute for Occupational Safety and Health. Criteria for a recommended standard for occupational exposure to beryllium. Washington D.C.: U.S. Government Printing Office, 1972.
14. U.S. National Institute for Occupational Safety and Health. National occupational hazard survey. Vol III. Washington D.C.: U.S. Government Printing Office, 1977. (Publication no. DHEW(NIOSH) 78–114).
15. Fabroni SM. Patologia polmonare da polveri di berillio. Med Lav 1935; 26:297–303.
16. Weber HH, Engelhardt WE. Untersuchung von stauben aus der berylliumsgewinnung. Zentralbl Gewerbehyg Unfallverhutung 1933; 10:41.
17. Caccuri S. Changes of liver and kidneys in beryllium intoxication experimental study of industrial toxic hepatonephritis. Rass Med Ind 1940; 11:307–14.
18. Voter SV. Toxicity of beryllium. Farmakol Toksikol 1940; 3:82–5.
19. Van Ordstrand HS, Hughes R, Carmody MG. Chemical pneumonia in workers extracting beryllium oxide. Cleve Clin Q 1943; 10:10–8.
20. Hardy HL, Tabershaw IR. Delayed chemical pneumonitis occurring in workers exposed to beryllium compounds. J Ind Hyg Toxicol 1946; 28:197.
21. Kress JE, Crispell KR. Chemical pneumonitis in men working with fluorescent powders containing beryllium. Guthrie Clinic Bull 1944; 13:91–5.
22. Machle W, Beyer E, Gregorius F. Berylliosis. Occup Med 1948; 5:671–83.
23. Anonymous editorial. Lancet 1951; 1:1357.
24. Stokinger HE, Sprague GF, Hall RH, Ashenburg NJ, Scott JK, Steadman LT. Acute inhalation toxicity of beryllium, I. Arch Ind Hyg Occup Med 1950; 1:379–97.
25. Stokinger HE, Ashenburg NJ, DeVoldre J, Scott JK, Smith FA. Acute inhalation toxicity of beryllium, II. Arch Ind Hyg Occup Med 1950; 1:398–410.
26. Hall RH, Scott JK, Laskin S, Stroud CA, Stokinger HE. Acute toxicity of inhaled beryllium, III. Arch Ind Hyg Occup Med 1950; 2:25–48.
27. Stokinger HE, Spiegl CJ, Root RE, *et al.* Acute inhalation toxicity of beryllium, IV. Arch Ind Hyg Occup Med 1953; 8:493–506.
28. Sanders CL, Cannon WC, Powers GJ, Adee RR, Meier DM. Toxicology of high-fired beryllium oxide inhaled by rodents. Arch Environ Health 1975; 30:546–51.
29. Reeves AL, Vorwald AJ. Beryllium carcinogenesis II. Pulmonary deposition and clearance of inhaled beryllium sulfate in the rat. Cancer Res 1967; 27:446–51.
30. Eisenbud M, Berghout CF, Steadman LT. Environmental studies in plants and laboratories using

beryllium: the acute disease. J Ind Hyg 1948; 30:281-5.

31. Sprince NL, Kazemi H. Beryllium Disease. In: Rom W, ed. Environmental and occupational medicine. 1st ed. Boston: Little, Brown, 1983; 481-90.

32. Freiman DG, Hardy HL. Beryllium disease: the relation of pulmonary pathology to clinical course and prognosis based on a study of 130 cases from the U.S. Beryllium Case Registry. Hum Pathol 1970; 1:25-44.

33. Andrews JL, Kazemi H, Hardy HL. Pattern of lung dysfunction in chronic beryllium disease. Am Rev Respir Dis 1969; 100:791-800.

34. Weber AL, Stoeckle JD, Hardy HL. Roentgenologic patterns in long-standing beryllium disease: report of 8 cases. AJR 1965; 93:879-90.

35. Aronchick JM, Rossman MD, Miller WT. Chronic beryllium disease: diagnosis, radiographic findings, and correlation with pulmonary function tests. Radiology 1987; 163:677-82.

36. Sprince NL, Kazemi H, Hardy HL. Current (1975) problem of differentiating between beryllium disease and sarcoidosis. Ann NY Acad Sci 1976; 278:654-64.

37. Cullen MR, Kominsky JR, Rossman MD, *et al.* Chronic beryllium disease in a precious metal refinery. Am Rev Respir Dis 1987; 135:201-8.

38. Klech H. Sarcoidosis: differential diagnosis. Semin Respir Med 1986; 8:72-94.

39. Lieben J, Metzner F. Epidemiological findings associated with beryllium extraction. Am Ind Hyg Assoc J 1959; 20:494-9.

40. Hasan FM, Kazemi H. Chronic beryllium disease: a continuing epidemiologic hazard. Chest 1974; 65:289-93.

41. Sprince NL, Kazemi H. U.S. Beryllium Case Registry through 1977. Environ Res 1980; 21:44-7.

42. Israel HL, Cooper DA. Chronic beryllium disease due to low beryllium content alloys. Am Rev Respir Dis 1964; 89:100-2.

43. Hooper WF. Acute beryllium lung disease. NC Med J 1981; 42:551-3.

44. Mark GJ, Monroe CB, Kazemi H. Mixed pneumoconiosis: silicosis, asbestosis, talcosis, and berylliosis. Chest 75:726-8.

45. Eisenbud M, Lisson J. Epidemiological aspects of beryllium-induced nonmalignant lung disease: a 30-year update. J Occup Med 1983; 25:196-202.

46. Kanarek DJ, Wainer RA, Chamberlin RI, Weber AL, Kazemi H. Respiratory illness in a population exposed to beryllium. Am Rev Respir Dis 1973; 108:1295-1302.

47. Sprince NL, Kanarek DJ, Weber AL, Chamberlin RI, Kazemi H. Reversible respiratory disease in beryllium workers. Am Rev Respir Dis 1978; 117:1011-7.

48. Kriebel D, Sprince NL, Eisen EA, Greaves IA. Pulmonary function in beryllium workers: assessment of exposure. Br J Ind Med (In Press).

49. Kriebel D, Sprince NL, Eisen EA, Greaves IA, Feldman HA, Greene RE. Beryllium exposure and pulmonary function: a cross-sectional study of beryllium workers. Br J Ind Med (In Press).

50. Sanders CL, Cannon WC, Powers GJ. Lung carcinogenesis induced by inhaled high-fired oxides of beryllium and plutonium. Health Phys 1978; 35:193-9.

51. Barna BP, Chiang T, Pillarisetti SG, Deodhar SD. Immunologic studies of experimental beryllium lung disease in the guinea pig. Clin Immunol Immunopathol 1981; 20:402-11.

52. Barna BP, Deodhar SD, Chiang T, Gautam S, Edinger M. Experimental beryllium-induced lung disease, I. Int Arch Allergy Appl Immunol 1984; 73:42-8.

53. Barna BP, Deodhar SD, Gautam S, Edinger M, Chiang T, McMahon JT. Experimental beryllium-induced lung disease, II. Int Arch Allergy Appl Immunol 1984; 73:49-55.

54. Schepers GWH, Durkan TM, Delahunt AB, Creedon FT. The biological action of inhaled beryllium sulfate. Arch Ind Health 1957; 15:32-58.

55. Hart BA, Harmsen AG, Low RB, Emerson R. Biochemical, cytological and histological alterations in rat lung following acute beryllium aerosol exposure. Toxicol Appl Pharmacol 1984; 75: 454-65.

56. Chiappino G, Cirla A, Vigliani EC. Delayed-type hypersensitivity reactions to beryllium compounds. Arch Pathol 1969; 87:131-40.

57. Votto JJ, Barton RW, Gionfrido MA, Cole SR, McCormick JR, Thrall RS. A model of pulmonary granulomata induced by beryllium sulfate in the rat. Sarcoidosis 1987; 4:71-6.

58. Hart BA, Pittman DG. The uptake of beryllium by the alveolar macrophage. J Reticuloendothelial Soc 1980; 27:49-58.

59. Camner P, Lundborg M, Hellstrom PA. Alveolar macrophages and 5 micrometer particles coated with different metals. Arch Environ Health 1974; 29:211-3.

60. Camner P, Hellstrom PA, Lundborg M, Philipson K. Lung clearance of 4 micrometer particles coated with silver, carbon or beryllium. Arch Environ Health 1977; 32:58-62.

61. Kang KY, Bice D, D'Amato R, Ziskind M, Salvaggio J. Effects of asbestos and beryllium on release of alveolar macrophage enzymes. Arch Environ Health 1979; 34:133-40.

62. Cullen MR, Cherniack MG, Kominsky JR. Chronic beryllium disease in the United States. Semin Respir Med 1986; 7:203-9.

63. Daniele RP, Elias JA, Epstein PE, Rossman MD. Bronchoalveolar lavage: role in the pathogenesis, diagnosis and management of interstitial lung disease. Ann Intern Med 1985; 102:93-108.

64. Daniele RP. Cell-mediated immunity in pulmonary disease. Hum Pathol 1986; 17:154-60.

65. Curtis GH. Cutaneous hypersensitivity due to beryllium. Arch Syphilol 1951; 64:470-82.

66. Reeves AL. Berylliosis as an auto-immune disorder. Ann Clin Lab Sci 1976; 6:256-62.

67. Deodhar SD, Barna B, Van Ordstrand HS. A study of the immunologic aspects of chronic berylliosis. Chest 1973; 63:309-13.

68. Price CD, Pugh A, Pioli EM, Jones Williams W. Beryllium macrophage migration inhibition test. Ann NY Acad Sci 1976; 278:204-11.

69. Hanifin JM, Epstein WL, Cline MJ. In vitro studies of granulomatous hypersensitivity to beryllium. J Invest Dermatol 1970; 55:284-8.

70. Williams WR, Jones Williams W. Comparison of lymphocyte transformation and macrophage inhibition tests in the detection of beryllium hypersensitivity. J Clin Pathol 1982; 35:684-7.

71. Jones Williams W, Williams WR. Value of beryllium lymphocyte transformation tests in chronic beryllium disease and in potentially exposed workers. Thorax 1983; 38:41-4.

72. Epstein PE, Dauber JH, Rossman MD, Daniele RP. Broncho-alveolar lavage in a patient with chronic berylliosis: evidence for hypersensitivity pneumonitis. Ann Intern Med 1982; 97:213-6.

73. Chihara J, Nagai S, Fugimura N, Hirata T, Izumi T. BAL lymphocyte findings in chronic beryllium disease. Am Rev Respir Dis 1983; 127(Suppl):64.

74. Rom WN, Lockey JE, Bang KM, Dewitt C, Johns RE. Reversible beryllium sensitization in a prospective study of beryllium workers. Arch Environ Health 1983; 38:302-7.

75. Jones JM, Amos HE. Antigen formation in metal contact sensitivity. Nature 1975; 256:499-500.

76. Resnick H, Roche M, Morgan WK. Immunoglobulin concentrations in berylliosis. Am Rev Respir Dis 1970; 101:504-10.

77. Vasilyeva EV, Nikitina LS, Orlova AA. Concentrations of immunoglobulins in berylliosis. J Hyg Epidemiol Microbiol Immunol 1977; 21: 254-60.

78. Reeves AL, Krivanek ND. The influence of cutaneous hypersensitivity to beryllium on the development of experimental pulmonary berylliosis. Trans NY Acad Sci 1974; 36:78-93.

79. Polak L, Barnes JM, Turk JL. The genetic control of contact sensitivity to inorganic metal compounds in guinea pigs. Immunology 1968; 14:707-11.

80. Vacher J. Immunological responses of guinea-pigs to beryllium salts. J Med Microbiol 1972; 5:91-108.

81. Stiefel T, Schulze K, Zorn H, Tolg G. Toxicokinetic and toxicodynamic studies of beryllium. Arch Toxicol 1980; 45:81-92.