Chronic Beryllium Disease in a Precious Metal Refinery

Clinical Epidemiologic and Immunologic Evidence for Continuing Risk from Exposure to Low Level Beryllium Fume [1-4]

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SUMMARY Five workers at a precious metal refinery developed granulomatous lung disease between 1972 and 1985. The original diagnosis was sarcoidosis, but 4 of the workers were subsequently proved to have hypersensitivity to beryllium by *in vitro* proliferative responses of lymphocytes obtained by bronchoalveolar lavage. Review of medical records of coworkers and extensive industrial hygiene surveillance of the plant demonstrated that 4 cases occurred in the furnace area where air concentrations of beryllium fume were consistently below the permissible exposure limit of 2 μg/M³. A single case has been recognized from parts of the refinery where exposures to cold beryllium dust often exceeded the standard by as much as 20-fold. These data demonstrate that chronic beryllium disease still occurs and confirm the importance of specific immunologic testing in patients suspected of having sarcoidosis but with potential exposure to beryllium. The data raise concern about the adequacy of modern industrial controls, especially in the setting of exposure to highly respirable beryllium fume.

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Introduction

Although 30,000 to 800,000 American workers have potential occupational exposure to beryllium or its compounds in the electronics, aerospace, metals, ceramics, and other industries (1), outbreaks or even cases of chronic beryllium disease (CBD) have been rarely identified since adoption by industry of voluntary environmental controls in the 1950s. Only 22 cases have been reported in workers who were first exposed after 1962, according to a recent epidemiologic review (2), and all were believed exposed to concentrations of beryllium higher than the permissible exposure limit of 2 μg/m³ (3). Several investigators have concluded that disease does not occur from low dose industrial exposures (2, 3), whereas others have stressed the possibility that sporadic cases may have been unrecognized because of the disorder's clinical and pathologic resemblance to sarcoidosis (4-6).

Prompted by the identification of a former worker at a metal refinery with suspect CBD, we undertook a clinical-epidemiologic investigation of the workplace and employees. By measuring *in vitro* proliferative response of lymphocytes to beryllium salts from bronchoalveolar lavage, recently suggested as a sensitive test for CBD (7-9), we have confirmed that an outbreak still can occur. Further, by extensive air sampling, we have been able to add additional information regarding the dose-response to beryllium, which has puzzled investigators for 4 decades.

Methods

Survey of the Work Force

The index case (Patient 1) was self-referred in 1980. Three additional patients with granulomatous lung disease meeting clinical-pathologic criteria of the beryllium case registry (5) (Patients 2 to 4) were identified by the index case and by inquiry through the patient's trade union. Subsequently, we attempted to define the population at risk and identify additional cases if present. All available active refinery workers were interviewed using a standard questionnaire to determine demographic background, job history, and symptoms. Medical records, spirometry, and chest radiographs that had been obtained routinely by the company within the previous 5 yr were reviewed. Additional testing was not performed. Unfortunately, data on laid-off, terminated, or retired workers were not available nor were the numbers of workers in the latter categories readily ascertainable. However, neither company nor union officials were aware of any men other than these 4 who left or were transferred from the refinery because of sarcoidosis or other lung disease.

A fifth worker, referred by a chest physician after local reporting of the cluster, developed disease subsequent to the survey (see below).

Industrial Hygiene

The evaluation consisted of collecting and analyzing 114 personal air samples measuring total airborne particulate. Sampling was roughly evenly distributed over job categories (see *Description of the Refinery Process* below) as well as over all work shifts. Multiple samples were obtained to characterize the range of exposures in an inherently variable "batch" process. The samples were obtained during 2 wk-long surveys, one in July and one in November 1983, to control for possible seasonal variation.

Each sample was collected for 7 to 8 h to define an 8-h time-weighted, average exposure to beryllium as well as to other toxic metals — arsenic, cadmium, lead, and nickel. The me-

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tals were collected on mixed cellulose ester filters 37 mm in diameter (0.8 μ nominal pore size) contained in a 3-piece, closed-face cassette using calibrated constant-flow sampling pumps operating at 2.0 L/min. The filters were analyzed in the laboratory of the National Institute for Occupational Safety and Health (NIOSH) using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) according to standard NIOSH protocol (11).

In addition to the personal monitors, 10 samples of settled dust were obtained from the crusher-screen, dry pan, and furnace areas for quantitative analysis. These samples were also analyzed by ICP-AES in the same laboratory.

Immunologic Studies

Patients 1 to 4 were studied at the Yale University School of Medicine Adult Clinical Research Center between 1983 and 1985. Patient 5 was lavaged as part of his initial diagnostic work-up at the Bridgeport (Connecticut) Hospital in 1985. After evaluation with chest radiographs and pulmonary function studies, each underwent bronchoalveolar lavage by fiberoptic bronchoscopy using a standard protocol (12). Briefly, lavage was performed as follows: after topical 4% xylocaine anesthesia was applied to the upper airway, an Olympus BF-1TR bronchoscope (Olympus Corp. of America, New Hyde Park, NY) was passed transnasally. Two to four 5-ml aliquots of 1% xylocaine were used endobronchially as needed to control cough. The middle lobe and lingula were then lavaged with 150 ml each of sterile, room-temperature saline. In addition, 15 ml of heparinized blood were obtained by venipuncture. This protocol had been approved by the Yale School of Medicine Human Investigation Committee, and prior written consent was obtained from all subjects.

Bronchoalveolar lavage was processed by first passing it through 1 to 2 layers of sterile gauze, followed by centrifugation at 500 *g* for 10 min at room temperature to pellet the cells. The lavage was decanted, and the cells were washed twice in modified calcium and magnesium-free Hank's balanced salt solution (KC Biological, Lenexa, KS). The cells were counted on a hemocytometer and assessed for viability with trypan blue dye exclusion. Differential cell counts were performed on Wright's stained cytocentrifuge preparations. The cells were prepared for transportation by resuspending them to a concentration of 1×10^6 cells/ml in RPMI 1640 supplemented with L-glutamine and buffered with Hepes and bicarbonate with 10% heat-inactivated normal human serum (MA Bioproducts, Walkersville, MD). They were placed in an insulated container at room temperature and shipped via Federal Express with overnight delivery. Peripheral blood mononuclear leukocytes were isolated by gradient centrifugation using Ficoll-Hypaque (12) and then processed similarly.

Lymphocytes were further analyzed in the pulmonary immunology laboratory at the University of Pennsylvania. Preliminary studies comparing cells prepared immediately and split samples isolated and stored overnight demonstrated that the lymphocytes processed in this manner maintain viability by trypan blue dye exclusion and *in vitro* responsiveness to mitogens and antigens. Unfortunately, the lavage sample from Patient 4 was inadvertantly processed differently, and this sample did not maintain adequate *in vitro* responsiveness to any mitogens. All transported peripheral blood samples maintained a degree of *in vitro* responsiveness comparable to fresh specimens.

The T-cell surface markers of blood and lavage lymphocytes were labeled with OKT_3, OKT_{11}, OKT_4, and OKT_8 (Ortho Diagnostics, Raritan, NJ), counterstained with goat antimouse immunoglobulin (TAGO, Burlingame, CA), and read on a fluorescence-activated cell sorter (Ortho-Spectrum III or Becton-Dickinson, Sunnyvale, CA) (13). Proliferative responses to standard mitogens (phytohemagglutinin, concanavalin A) and beryllium salts (fluoride and sulfate, diluted to 10^{-4}, 10^{-5}, and 10^{-6} M) *in vitro* were assayed by measurement of incorporation of tritiated thymidine by the method previously published (7). Response was expressed as a peak stimulation index: the highest count of labeled thymidine measured on Day 3, 5, or 7 taken up by mitogen- or beryllium-stimulated (any dilution) lymphocytes divided by concurrently measured uptake in cells cocultured without the additive. Isolated elevations not corroborated by all responses at other dilutions and times were disregarded. A stimulation index of greater than 5 on a minimum of 2 cultures was considered a positive biologic response based on the laboratory experience with these and subsequent cases and control specimens (see RESULTS).

In addition to those from the 5 study cases, blood and bronchoalveolar lavage were obtained at the Hospital of the University of Pennsylvania from 10 patients with sarcoidosis, none of whom had known exposure to beryllium. Identical lymphocyte proliferation studies were performed on these cells during the same time period. (8).

Normative data on cellular material in bronchoalveolar lavage were obtained on 19 healthy, nonsmoking, control subjects studied at Yale-New Haven Hospital.

Additional data from patients with sarcoidosis studied at Yale-New Haven Hospital were used. None had a history of exposure to beryllium (12).

Case Reports

Patient 1 (index case). This 40-yr-old, nonsmoking Puerto Rican man referred himself to the Yale Occupational Medicine Clinic in 1980 for suspected "berylliosis." In 1964, he had moved to Connecticut and taken employment at the refinery; he was stationed at the furnace. In 1972, he developed chronic respiratory symptoms. Despite transfer out of the refinery, symptoms progressed. In 1976, he was hospitalized for evaluation. A chest radiograph (figure 1) showed hilar adenopathy and diffuse parenchymal infiltrates bilaterally. Lung function studies revealed a restrictive defect with a decreased diffusing capacity for carbon monoxide. Open lung biopsy

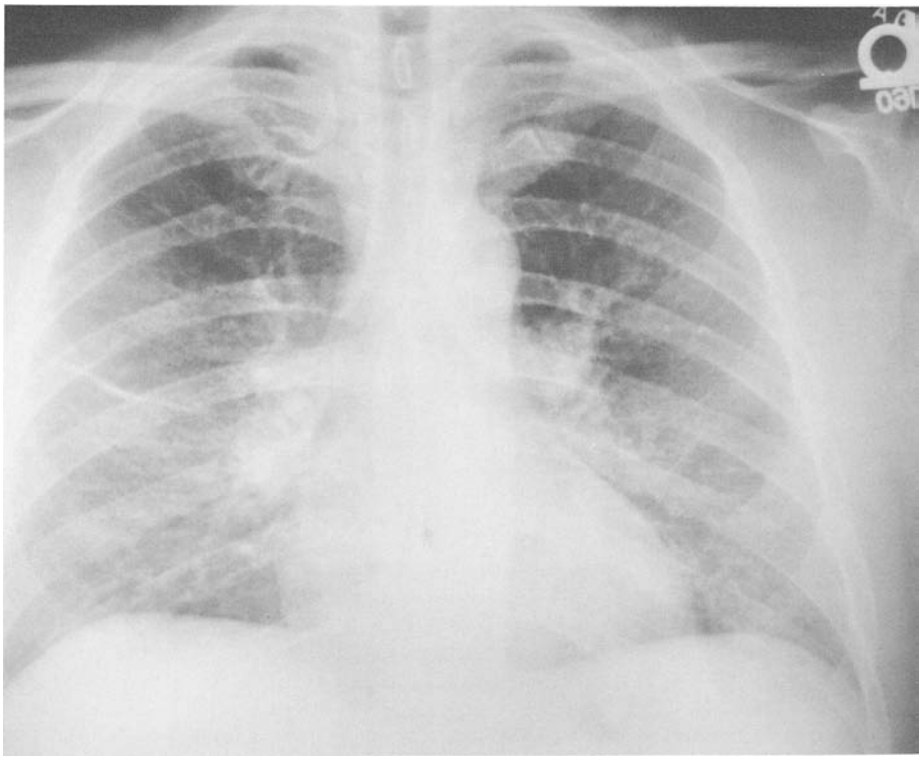

Fig. 1. Posteroanterior view of the chest of Patient 1 showing bilateral hilar lymphadenopathy and diffuse symmetric interstitial infiltrate.

(figure 2) revealed noncaseating granulomata and pulmonary fibrosis. Stains and cultures for mycobacteria and fungi were negative. Because of his occupation, 24-h urine specimens were collected to test for beryllium; none was detected. He was begun on corticosteroids for presumed sarcoidosis.

Between 1976 and 1980, the patient independently investigated a possible relationship between exposure at the furnace and his lung disease, prompted by the occurrence of "sarcoidosis" in several coworkers. He maintained a list of raw materials delivered to the receiving room of the plant; many contained beryllium alloys.

Reevaluation in 1980 showed diffuse dry crackles over the chest and moderate digital clubbing; he was slightly tachypneic at rest. Dermatologic and general physical examination was otherwise unrevealing. A chest radiograph and lung function tests (FVC = 2.76 L, 4.27 predicted; FEV_1/FVC = 82%; D_{LCO} = 17.6 ml/min/mm Hg, 29.2 predicted) were unchanged from 1976 despite discontinuing steroids after 1 y. Complete blood count and differential, serum electrolytes, urea nitrogen, creatinine, AST, alkaline phosphatase, bilirubin, calcium, uric acid, and angiotensin-converting enzyme determinations were all normal. Ashing of his prior lung specimen for beryllium content demonstrated 0.016 μg/g dry weight, a high normal concentration (10).

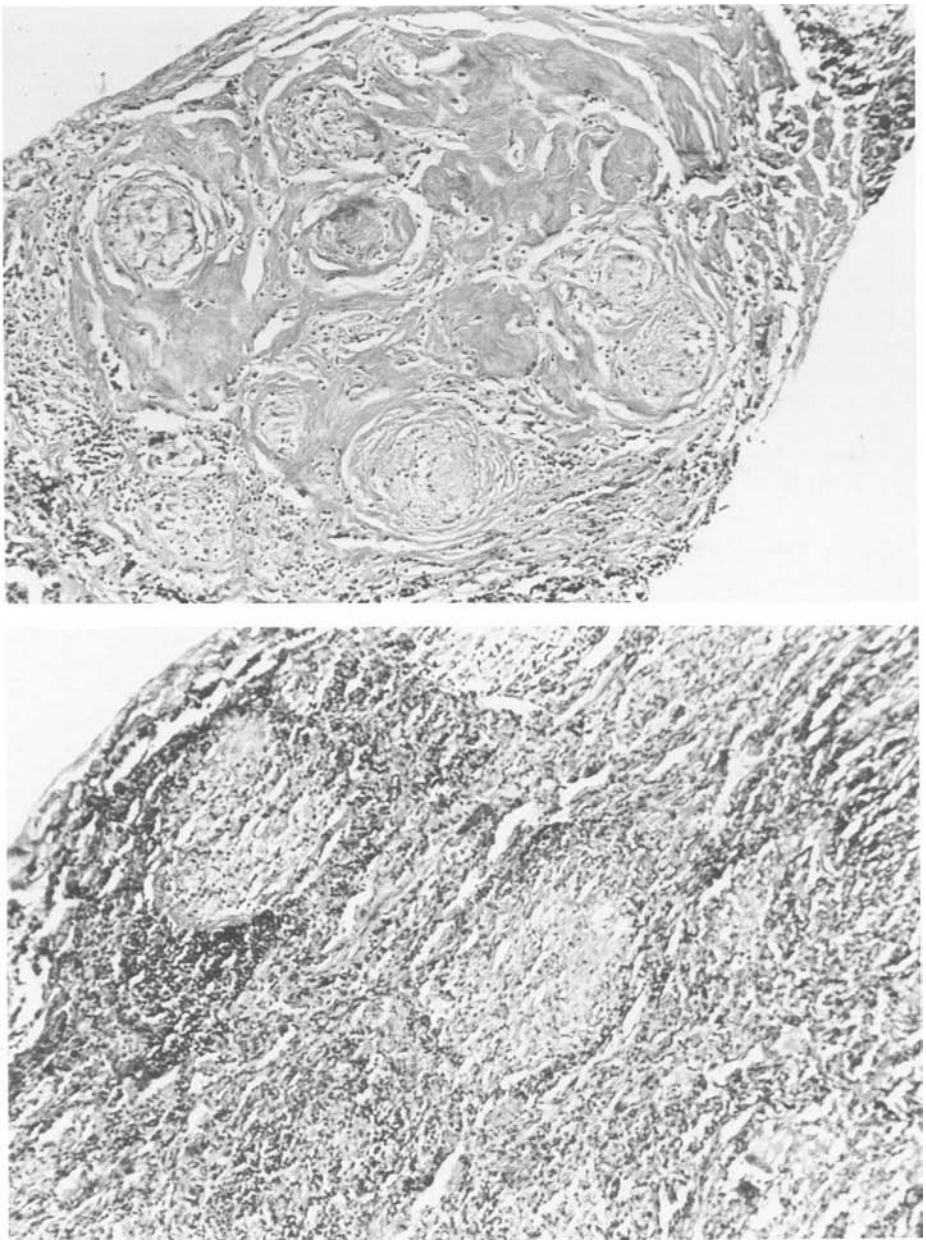

Fig. 2. A. Open lung biopsy of Patient 1. Hemotoxylin-eosin stain; magnification: ×100. Granulomatosis diagnosed on lung tissue after biopsy. Multiple epithelioid granulomas that are becoming hyalinized are surrounded by dense fibrosis and sparse population of lymphocytes. Lesion is similar to that seen in aging lesions of idiopathic sarcoidosis. B. Hemotoxylin-eosin stain; magnification: ×100. Elsewhere, lung architecture is obscured by epithelioid granulomatosis without necrosis. Lung interstitial space and alveoli are filled with a lymphohistiocytic infiltrate. Microscopic features suggest hypersensitivity lung disease in addition to sarcoidosis.

Patient 2. This 31-yr-old Puerto Rican man began work at the refinery in 1971 when he came to Connecticut, but he never worked near the index case. He smoked less than 10 cigarettes a day. In 1975, he developed respiratory and constitutional symptoms. Evaluation in 1976 revealed an abnormal chest radiograph with hilar adenopathy and reticulonodular infiltrates. Lung function showed normal lung volumes and flow rates; D_{LCO} was 50% of predicted. A percutaneous liver biopsy showed noncaseating granulomas; AFB and fungal stains and cultures were negative. Sarcoidosis was diagnosed. No therapy was begun. Because of his symptoms, the patient was transferred out of the refinery area.

Reevaluation in 1980 showed little progression clinically. Crackles were auscultated over the lung fields; the remainder of the physical examination was normal. Blood count, differential, urinalysis and serum electrolytes, urea nitrogen, creatinine, calcium, AST, alkaline phosphatase, bilirubin, uric acid, and angiotensin-converting enzyme determinations were normal. Radiographs and lung function studies (FVC = 3.88 L, 3.60 predicted; FEV_1/FVC = 79%; D_{LCO} = 17.1 ml/min/mm Hg, 30.1 predicted) were unchanged.

Patient 3. This 35-yr-old Portuguese man has been in good health prior to starting work as a furnace operator in 1972; he was a nonsmoker. He had been told of possible hilar enlargement while in military service 10 yr earlier. In 1977, he developed a respiratory illness with persistent fevers and arthralgias. Radiographs showed moderate hilar enlargement and diffuse bilateral parenchymal infiltration. He had reduced lung volumes and marked impairment of diffusing capacity. Noncaseating granulomas without fungi or acid-fast bacilli were demonstrated by transbronchial biopsy. Steroids were begun, and he was transferred out of the refinery area.

In 1980, he was stable functionally (FVC = 3.21 L, 4.89 predicted; FEV_1/FVC = 86%; D_{LCO} = 18.0 ml/min/mm Hg, 31.1 predicted) and radiographically unchanged, although he remained on corticosteroid therapy. Physical examination, blood count, and urinalysis and serum urea nitrogen, electrolytes, AST, alkaline phosphatase, bilirubin, calcium, and angiotensin-coverting enzyme determinations were normal.

Patient 4. This 41-yr-old nonsmoker had moved from rural Puerto Rico to Connecticut in 1968 where he began work in the furnace area of the refinery; he had no prior industrial work history or exposure to known respiratory toxins. In 1972, he developed an illness characterized by fevers, cough, and dyspnea. During hospital evaluation, he was reported to have eosinophilia and abnormal hepatic function as well as hilar lymphadenopathy and advanced pulmonary parenchymal infiltration with fibrocystic changes. Liver biopsy revealed noncaseating granulomas and

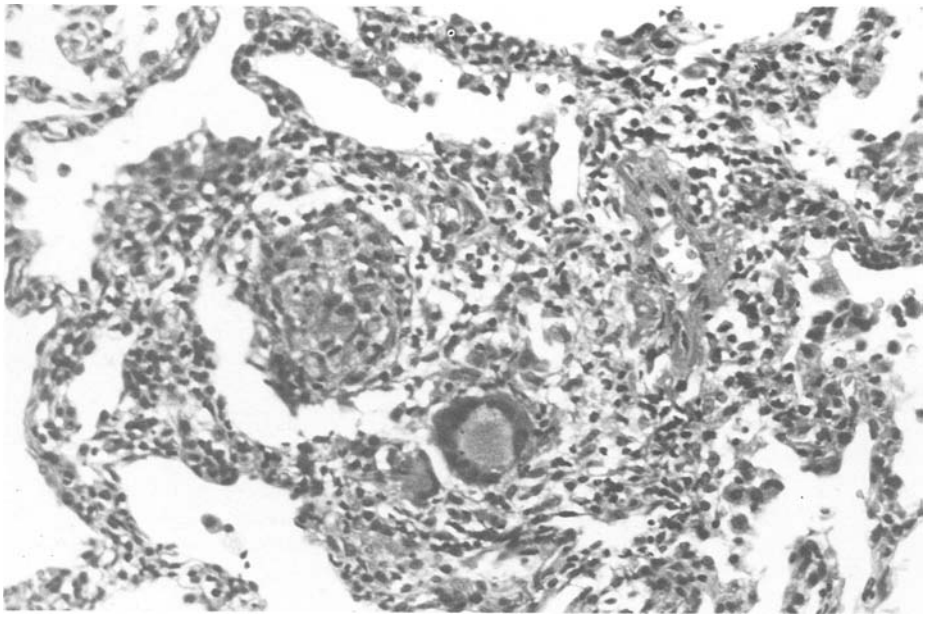

Fig. 3. Open lung biopsy, Patient 4. Hematoxylin-eosin stain; magnification ×250. Epithelioid granulomatosis with multinucleated giant cells. Lymphocytic interstitial infiltrate and lymphocytic alveolitis was moderate in this patient. Fibrosis was minimal at the time of this biopsy.

ova of *Schistosoma mansoni*. Open lung biopsy revealed diffuse noncaseating granuloma, Schauman bodies, and fibrosis. Ova, eosinophils, and vascular involvement often seen in association with granulomas in pulmonary schistosomiasis were notably absent on the lung specimen (figure 3). Cultures and special stains for mycobacteria and fungi were negative. He was discharged with the diagnosis of gastrointestinal Schistosomiasis and probable sarcoidosis. Corticosteroids were begun and the patient shortly thereafter returned to Puerto Rico.

Reevaluation in 1980 revealed a chronically ill, cushingoid man in moderate respiratory distress. Physical examination was remarkable for diffuse wheezing and an enlarged liver. Fingers were moderately clubbed. Serum AST, ALT, and alkaline phosphatase determinations were mildly elevated. Peripheral blood count and smear and angiotensin-converting enzyme determinations were normal. His chest radiograph is shown in figure 4; the original films were not available for comparison. Pulmonary function tests showed a FVC of 2.88 L, with 4.51 predicted. The FEV_1/FVC was 73%; single-breath D_{LCO} was 13.1 ml/min/mm Hg with 28.9 predicted.

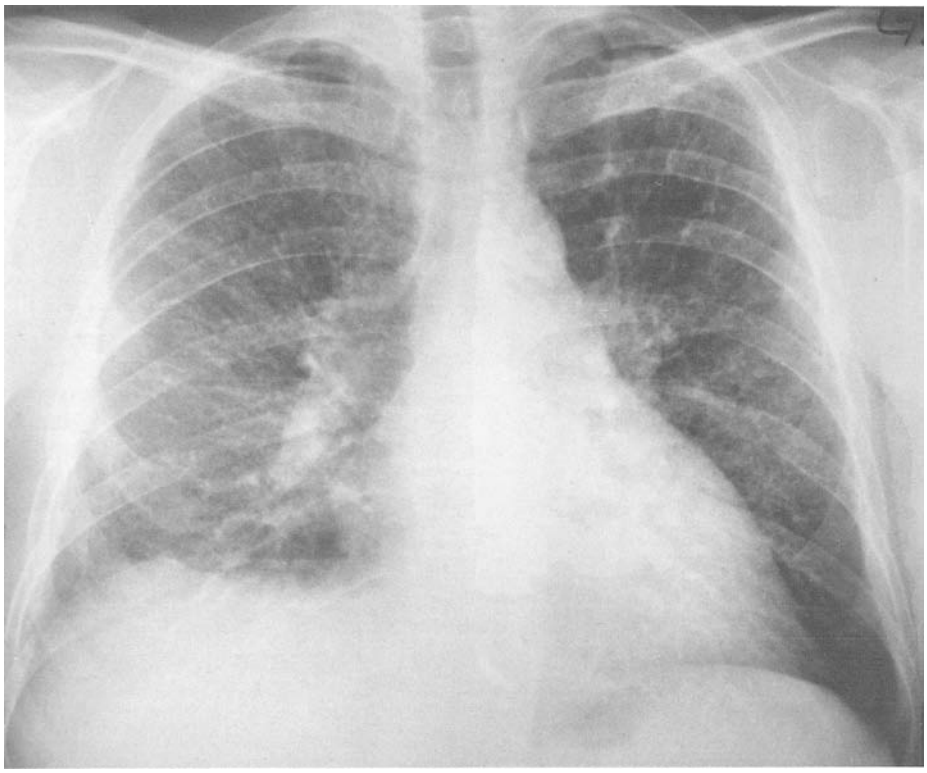

Fig. 4. Posteroanterior radiograph of Patient 4 obtained in 1980 during first Yale visit. Bilateral hilar enlargement is seen with diffuse reticulonodular infiltrate. Fibrocystic changes are seen in the left upper lung zone. The right hemidiaphragm is raised. There is bilateral pleural thickening and borderline cardiomegally.

Patient 5. This 38-yr-old black man had worked as a crusher/separator from 1969 until 1980 when he transferred to the mixer/sampler area; he participated in the 1983 survey of refinery workers and had an unremarkable clinical history and prior radiographs. In mid-1984, he developed arthralgias of the ankles but did not seek attention. In December 1984, he noted onset of exertional dyspnea and weight loss. He was a light smoker and former heavy beer drinker. On evaluation in April 1985, he was noted to have a normal physical examination except for an old keloid scar on the chest and inguinal lymphadenopathy; CBC and urinalysis were normal. Serum ALT, AST, and alkaline phosphatase determinations were mildly elevated; there was a polyclonal increase in serum gamma globulin. A chest radiograph showed diffuse interstitial infiltrate and right hilar adenopathy; and a CT scan of the chest demonstrated enlarged hilar nodes bilaterally, as well as paratracheal and subcarinal node enlargement. Lung function tests revealed mild reduction of lung volumes (FVC = 3.47 L, 4.87 predicted), normal flow rates, and D_{LCO} only 9.7 ml/min/mm Hg, with 30.7 predicted. Transbronchial biopsy of right lower and middle lobes revealed noncaseating granulomas, giant cells, and moderate alveolitis; mycobacteria and fungi were excluded. Chronic beryllium disease was diagnosed based on lavage studies, and corticosteroids were begun.

Description of the Refinery Process

The company is engaged in the refining and reclamation of precious metals from industrial scrap using waste materials culled from the electronics, computer, photographic, chemical, and decorative industries; many contained beryllium compounds or alloys. Homogeneous metal ingots and powders of varying purities of gold and silver are produced.

The process is diagrammed in figure 5. The materials are physically described and weighed in the receiving room and classified according to the flow process best suited for refining. Wet materials and those with organic constituents (e.g., circuit boards) are first incinerated for drying and elimination of carbonaceous material. The residue then goes through mechanical processing including crushing, ball milling, and screening. Large particles are sent to one of several furnaces for melting and are subsequently cast as 1,000-ounce ingots. Small particles are blended with other powders to produce desired purities.

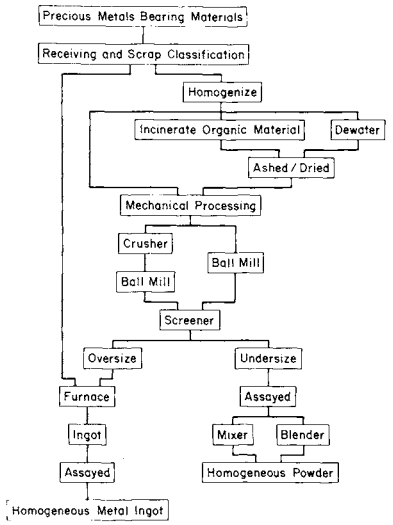

Fig. 5. Schematic flow chart of the refinery process.

These powders and ingots are shipped to other areas for further processing and finishing.

The refinery work force of approximately 70 is divided into 6 distinct job groupings: samplers, crusher-screen operators, ball-mill operators, floor sweepers, furnace tenders, and dry pan operators. With the exception of some utility workers who rotate jobs, assignments tend to be stable with infrequent switching from category to category. Job turnover is low because of a union wage scale and benefits.

On the basis of extensive interviewing with management and workers by the investigating industrial hygienist (JRK), it was determined that the process and ventilation had been largely unchanged for decades. Beginning in 1981, all refinery workers were required to wear air-purifying respirators. No respiratory protective devices had been routinely used prior to that date.

Results

Survey of the Work Force

Excluding the previously recognized workers (Patients 1 to 4) who had been transferred from the refinery at the time of the survey, 45 of 70 current refinery workers were available for questioning; others were unavailable because of temporary layoff, transfer, or illness. Their mean age was 44.5 yr. Average duration of employment at the facility was 14.4 yr, with an average of 12.6 yr in the refinery. Ethnic composition was 31% northern European, 33% Portuguese, 16% Hispanic, and 20% black.

Review of specific job assignments within the refinery revealed that 29% were currently assigned to jobs that involved some work around the furnaces (including utility workers and repairmen). Workers had changed jobs on the average of once every 4 yr, although most changes were within rather than between job groupings. Overall, 38% of the group had had at least one assignment around the furnaces.

One third of the refinery workers presently worked in mechanical processing as ball mill operators, crushers, or samplers. Almost half had worked in mechanical processing at some time while at the refinery.

Clinical assessment by questionnaire and review of prior radiographs and spirometry results was undertaken for evidence of granulomatous lung disease. Eighteen current workers had lower respiratory complaints (cough, dyspnea, or wheezing) believed to have occurred since onset of employment. Of these, 7 had either abnormal spriometry results or nonspecific radiographic abnormalities (air trapping, bullae, or focal scarring). No current refinery worker, however, had either a radiographic or spirometry result suggestive of sarcoidosis or chronic beryllium disease.

Industrial Hygiene

Time-weighted average personal air samples for beryllium throughout the refinery revealed a wide range of values from 0.22 to 42.3 μg/m³ (mean, 1.2 ± 0.96 μg/m³). These values closely fit a log-normal distribution ($R > 0.9$); 10% of all samples were in excess of the present permissible exposure limit of 2.0 μg/m³ (14).

Moderate concentrations of other metal contaminants were also detected in the samples as follows (mean ± SD in μg/M³): arsenic, 0.82 ± 0.26; cadmium, 38.9 ± 27.2; lead, 20.3 ± 15.2; nickel, 91.9 ± 67.6.

Analysis of air samples by job classification revealed striking differences. Beryllium concentrations for furnace tenders, sweepers, and dry pan operators were uniformly below the standard, with means of 0.52 ± 0.44, 0.63 ± 0.32, and 0.46 ± 0.48 μg/m³, respectively, whereas those for the crushers and ball mill operators were much higher (mean, 2.7 ± 7.2 and 2.1 ± 1.6 μg/m³); samplers were intermediate (mean, 0.72 ± 0.95). These data are shown in figure 6. Concentrations of the other materials had a distribution by job category quite similar to that of beryllium.

Although the airborne metal in the furnace area represented primarily fume (oxides produced by heating), whereas that in other areas was primarily metal alloy dust (from mechanical manipulations), the beryllium fraction, as measured by the settled dust samples, was virtually

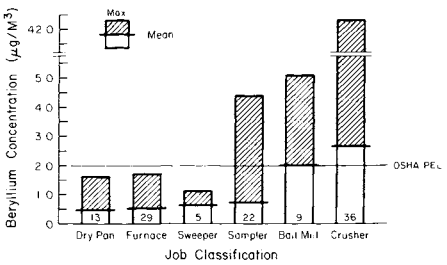

Fig. 6. Mean and maximal personal sample concentrations for workers in the 6 job categories. All samples are 8-h time-weighted average values. Numbers in bars represent the total number of samples taken for each category (OSHA PEL = permissible exposure limit of the Occupational Safety and Health Administration).

identical throughout the shop (0.04 ± 0.01% by weight). Contamination with other metals was also consistent throughout the shop, although higher cadmium content was noted at the dry pans. Neither particle sizes nor actual chemical composition (e.g., oxide versus metal) were assessed directly.

Immunologic Studies

Four of the patients from the plant had increased cell counts in lavage fluid with a mean return for all of 76.6 million total cells (lab normal for nonsmokers = 15.9 ± 9.3 million) (12). Differential counts revealed marked lymphocytosis in Patients 1, 2, 3, and 5. Patient 4 had a predominant eosinophilia. These data are demonstrated in table 1.

Monoclonal antibody studies to determine phenotype were performed on blood lymphocytes of all patients and lavaged lymphocytes from all patients except Patients 3 and 4. As noted above, the lavage sample of Patient 4 was inadvertantly processed and transported by a different method, and the lymphocytes were subsequently nonviable. Results of these studies are shown in table 2.

Proliferation of blood and lavage lymphocytes in response to mitogens and beryllium salts was assayed and quantified as a peak stimulation index, the ration of maximal uptake of tritiated thymidine in the patient's cells cultured with the mitogen to uptake of cells cultured without the mitogen. All patients showed strong positive peripheral blood responses to phytohemagglutinin and concanavalin A. Blood lymphocytes from Patient 1 only, however, showed a clearly positive response to beryllium salts, with a peak stimulation index of 6.6; Patients 2 and 3 showed a slight response (peak stimulation indexes = 2.4 and 4.9, respectively), Patients 4 and 5 showed no response. Absolute thymidine

TABLE 1

TOTAL CELLULARITY OF BRONCHOALVEOLAR LAVAGE AND DIFFERENTIAL CELL COUNTS

Patient No.	Δ to Total Cells (*in millions*)	Alveolar Macrophages (%)	Lymphocytes (%)	Polymorphonuclear Leukocytes (%)	Eosinophils (%)	Basophils (%)
1	183	17.8	80.9	0.2	1.0	0.1
2	81.3	31.2	58.2	4.1	6.0	0.5
3	54.5	42.3	57	0.3	0.4	0
4	47	30.5	12	1.2	55.5	0.8
5	17	30	70	0	0	0
Normal subjects*	15.9 ± 9.3	88.3 ± 1.7	11.0 ± 1.7	0.56 ± 0.1	0.06 ± 0.1	0.06 ± 0.03

* Mean ± SD, n = 19.

uptake in unstimulated cultures ranged from 180 to 3,000 cmp.

Lavage lymphocytes from Patients 1, 2, 3, and 5 showed a positive response to nonspecific mitogens (peak stimulation indexes for PHA: 24, 27, 13.3, and 2.6, respectively; for Con A: 28, 5.1, 12.4, 9.1) with generally more marked responses to beryllium salts (beryllium peak stimulation indexes: 58, 62, 29.4, and 7.8, respectively). Unstimulated cultures for the patients ranged between 90 and 570 cpm.

By contrast, although blood and lavage lymphocytes from all control subjects with sarcoidosis responded to nonspecific mitogens, responses to beryllium stimulation were minimal, with a mean peak index on lavage cultures of 3.2 and a SD of 1.2. Five had peak stimulation ratios between 3.0 and 4.6; an isolated measurement of 5.3 in 1 control subject was unsubstantiated by any other preparation. Blood responses in these subjects were in the same range; 2 had isolated peaks exceeding 5. The results of the responses of lavage lymphocytes to beryllium salts for patients and sarcoidosis control subjects are displayed in figure 7. A detailed description of responses of blood and lavage lymphocytes to mitogens and specific antigens in chronic beryllium disease and sarcoidosis, and in normal subjects will be presented in a subsequent report.

Discussion

Several distinct issues regarding the occurrence, cause, and diagnosis of chronic beryllium disease have been addressed by these clinical-epidemiologic, immunologic, and environmental investigations. Although alternative interpretations exist for certain aspects of the data, we believe there is sufficient information to resolve some major outstanding controversies.

First and most importantly, we believe there is now compelling evidence that chronic beryllium disease mimicking sarcoidosis still occurs from modern industrial exposures. There would appear to be little doubt that Patients 1, 2, and 5 have CBD. Each has histologically confirmed granulomatous disease, documented beryllium exposure, and marked *in vitro* proliferation of lung lymphocytes to beryllium salts. Patient 3 meets these criteria, but the history of possible hilar enlargement in 1962 (before beryllium exposure) raises the possibility of coincidental beryllium sensitivity in a man with active alveolitis caused by sarcoidosis. However, the history of symptom onset in 1977 and the absence of hypersensitivity in previously reported beryllium workers without chronic beryllium disease (9) or in patients with sarcoidosis (figure 7) makes this unlikely. Despite meeting the published clinical criteria for chronic beryllium disease (5), Patient 4 is more problematic in view of coexistent gastrointestinal schistosomiasis, predominant eosinophilia on bronchoalveolar lavage, negative peripheral blood testing, and our failure to obtain viable lavage lymphocytes. Overall, then, probably 4 and possibly 5 cases of chronic beryllium disease have been identified in a single small shop.

Although the precise number of workers at risk in this facility during the period is unclear because of lack of data on terminated, transferred, and retired work-

TABLE 2

PROPORTIONS OF LYMPHOCYTE PHENOTYPES ON PERIPHERAL BLOOD AND BRONCHOALVEOLAR LAVAGE CELLS FROM PATIENTS AND CONTROL VALUES FROM HEALTHY NORMAL SUBJECTS AND PATIENTS WITH SARCOIDOSIS

	Peripheral Blood				Bronchoalveolar Lavage			
Patient No.	Total T cells	T4	T8	T4/T8	Total T cells	T4	T8	T4/T8
	(% *Lymphocytes*)				(% *Lymphocytes*)			
1	68.1†	27.4	42.8	0.64	96†	76	17.9	4.2
2	72.1‡	11.1	29.4	0.45	76.9‡	83.1	8.2	10.1
3	72.8†	19.8	36.1	0.55	ND	ND	ND	ND
4	80.0§	49.4	45.4	1.08	ND	ND	ND	ND
5	47.9†	5.6	27.5	0.20	89.5	88.5	18	4.9
Patients with sarcoidosis¶	76.0 ± 2.8	54.6 ± 3.2	35.6 ± 3.5	2.3 ± 0.8	87.8 ± 1.0	75.0 ± 1.9	25.1 ± 2.5	4.2 ± 0.5
Healthy normal subjects‖ Mean ± SE	85.3 ± 2.4	56.0 ± 2.0	37.1 ± 1.8	1.5 ± 0.1	78.6 ± 2.9	55.1 ± 3.2	38.2 ± 2.0	1.5 ± 0.01

† Using T11 monoclonal antibody.
‡ Using T3 monoclonal antibody.
§ Using neuraminadase-treated sheep red blood cell rosette isolation.
¶ Mean ± SD; n = 28.
‖ Mean ± SD; n = 7.

ers, the number is unlikely to exceed twice the present 70 workers, given the employment pattern described above. Assuming, conservatively, no further cases among those who have left, this yields a cumulative disease prevalence between 2.9 and 3.6%. In view of other recent reports of new cases, also initially considered sarcoidosis, from the aircraft industry (15) and secondary copper smelting (16), it appears likely that recent predictions of the disappearance of chronic beryllium disease (2) have been premature. Although industrial hygiene has undoubtedly decreased risk, misdiagnosis and underreporting probably explain at least part of the apparent decline in the incidence of the disease.

A second major finding of the study is the demonstration of chronic beryllium disease in workers exposed to concentrations of beryllium apparently below the permissible exposure limit of 2 μg/M³ (Patients 1 to 4, each of whom worked primarily at the furnace area prior to disease onset). No cases from such low level industrial exposure have ever been reported (2, 3), although neighborhood cases have been recognized after apparently much lower level exposures (17).

Several considerations may limit the serious implication of this finding. First, the standard filter method of collection may underestimate total beryllium at the furnace where beryllium is likely to be in the form of a fine fume, unlike the dust exposures elsewhere in the refinery. Fine particles less than the membrane filter size, therefore, could be missed. Second, since exposures occurred for these patients between 1964 and 1977, whereas sampling was performed in 1983, historic concentrations may have been higher than those measured. Alternatively, it might be that insufficient samples were taken to fully characterize even the present exposure conditions; batches with much higher beryllium content may have been missed. Fourth, since all of the patients also had simultaneous exposures to cadmium, nickel, lead, and arsenic, it is possible that these agents may be altering the attack rate or pattern of disease. Finally, since concentrations outside the furnace area were, indeed, often much higher than the standard, it might be the case that brief periods of time spent away from the primary job assignment (for example, during overtime), not exposures at the furnace *per se*, were causal.

Although certainly plausible, we believe that none of these possibilites is very likely. Although it is possible that standard methods of collection underestimate total beryllium, this effect is unlikely to be great. The efficiency of the filter down to 0.8 μ is virtually 100%. Smaller particles are also largely captured by electrostatic forces and impaction on the walls of pore passages formed by the interlocking of cell layers. Those that are missed, furthermore, are unlikely to significantly affect mass since they are so small, mass varying as a third power of diameter.

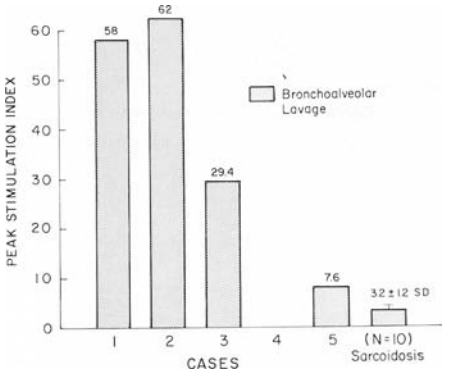

Fig. 7. Maximal proliferative response to beryllium of bronchoalveolar lavage lymphocytes for study cases expressed as stimulation index. Unstimulated cultures had absolute uptakes of 90 to 570 cpm. Lavage lymphocytes of Patient 4 did not respond adequately to any mitogen. Control subjects with sarcoidosis are presented as mean and standard deviation of peak proliferative responses. No peak response was confirmed greater than 5, which is considered positive (see text).

Regarding historical exposures, a very careful survey of plant furnaces and ventilation, as well as interviews with management and workers, revealed that virtually no structural changes nor change in work practices had in fact occurred during the 20 yr since the index case first took employment there. A single new furnace area installed since 1978 was not being used during the air-monitoring survey, and thus was not sampled. A more relevant possibility for historical changes in beryllium concentrations or composition would be from changes in the composition of the scrap purchased by the company. However, plant records show increasing purchase of electronic parts during the past 10 yr, suggesting that present concentrations may be, if anything, higher on average than those of previous years. Therefore, although the possibility certainly exists that exposures may have been higher in the past, there is no available evidence to suggest this.

Regarding the adequacy of sampling, we believe that the 29 full-shift samples taken at the furnaces during 2 separate week-long surveys (1 in July, 1 in November) over all shifts, were highly representative. That scrap batches samples contained high beryllium content was confirmed by the very high concentrations simultaneously measured in other parts of the operation (figure 6). There was no evidence of limited, altered, or atypical work practices during the survey. Furthermore, the characteristic log normal probability distribution of sample values obtained suggests that it is unlikely that many widely deviant air concentrations were "missed".

Concerning the role of coexposure to metals other than beryllium, only cadmium exposures were comparable at the furnace with those in other parts of the plant, the others being much lower there. Although this toxic metal may cause chronic respiratory injury or alter metabolic clearances of other metals (18), there is no evidence that it induces or enchances granuloma formation, nor that it has a specific interaction with the structurally unrelated metal beryllium.

Could brief nonfurnace exposures to higher beryllium concentrations in metal dust from neighboring mechanical operations have caused sensitization? This possibility appears unlikely from the clinical-epidemiologic data. If such exposures were indeed more pathogenic than the furnace work, it would seem improbable that as many as 4 cases would have occurred among the one third of men who worked predominantly at the furnaces while only 1 occurred in the one third of the group working primarily in these high exposure areas (i.e., samplers, crushers, and ball mill operators).

In fact, using the same reasoning, the data from our study suggest the reverse—that fume from high temperature operations is more pathogenic than metal dust despite the far higher air concentrations of the latter. This would be true even if historic concentrations of beryllium were higher than those measured given the apparently dustier nature of the "cold" milling operation. Correcting for differences in dose, a substantial differential risk between fume and metallic dust is demonstrated. This is consistent with the historically recognized fact that beryllium disease from "cold-working" of alloys rarely occurs (2). To our knowledge, however, this refinery is the first site where the differential risk has been demonstrated in a worker population at a single facility.

Putting the data together, it is the conclusion of our study that significant risk for chronic beryllium disease exists among that portion of the 30,000 to 800,000 American workers exposed to

beryllium, especially those who smelt, burn, refine, or weld the metal or its alloys, possibly even if exposure concentrations are below presently adopted standards. On the basis of our experience and that recently reported, cases will most likely present in a fashion indistinguishable from sarcoidosis. Use of *in vitro* proliferation testing on lymphocytes obtained by bronchoalveolar lavage appears to offer a reliable test for differentiation in most cases (8, 9). Given the potential clinical, social, and public health implications of correct diagnosis, physicians should closely question all patients with suspect or proved granulomatous lung disease for possible past or present exposure to metals, alloys, or other potentially beryllium-containing materials and have a low threshhold for undertaking the suggested differentiating studies. Perhaps more importantly, further investigations should be performed to reassess the validity of the presently allowable air standards where workers may be exposed to respirable fume if additional cases and outbreaks of chronic beryllium disease are to be prevented in the future.

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