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DEMONSTRATION OF THE PRESENCE OF BERYLLIUM IN PULMONARY GRANULOMAS

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Although there are approximately 700 cases of human berylliosis on file in the Beryllium Case Registry,⁵ the absolute diagnosis of many of these remains in doubt as a result of the lack of a sufficiently reliable and sensitive method for detecting the presence of beryllium in either biopsy or postmortem tissues and for correlating its presence with specific anatomic changes. In those instances where beryllium has been successfully recovered from pulmonary tissues by various chemical methods,^{1-4, 6, 7, 9} there still remains the problem of direct association with pathologic lesions, as the methods *per se* have destroyed the structural integrity of the analyzed tissue. Nor has there been available a suitable histochemical procedure for identification of beryllium in specific tissue sections.

Only within the past few years have beryllium-induced granulomas been successfully produced in monkeys and rats,^{8, 9, 11} in order that the disease entity might be studied experimentally. With the advent of the laser microprobe and emission spectroscopy, we believed that this approach to the

demonstration of beryllium in pulmonary granulomatous lesions would be worthy of investigation.

Our study was initiated in 1961, when dogs were experimentally exposed to respirable dusts containing beryllium. These animals were maintained under long-term observation in our laboratories and were subjected to necropsy. Microscopic examination of tissues revealed pulmonary lesions characteristic of beryllium granulomas; these lesions were analyzed for beryllium content by micro-emission spectrography.

MATERIALS AND METHODS

Two groups of purebred beagle dogs, 9 months old, were exposed by the natural respiratory route, without cannulation, to dusts containing beryllium. Group 1 consisted of 3 males and 3 females, from 2 different litters and weighing from 7.3 to 10.8 kg., with a mean weight of 9.1 kg. Two males, 1 from each litter, weighing 9.5 and 10.4 kg., were used as unexposed controls. Two additional dogs, 1 male and 1 female, completed Group 2. They weighed 10.8 and 8.1 kg., respectively. The 6 dogs in Group 1 were exposed to a dust of nonhalogenated beryllium, primarily beryllium oxide, at an average concentration of 120 mg per m.³ (range 40 to 300 mg. per m.³) for 20 min. The 2 beagles in Group 2 were exposed to dust of halogenated beryllium material, at an average concentration of 115 mg. per m.³, also for 20 min. The unexposed control dogs received the same kennel maintenance and veterinary care as the exposed animals. All dogs were maintained in a vivarium and were periodically weighed and carefully examined for signs of clinical illness. The animals were subsequently sacrificed by means of an overdose of intravenous barbitol anesthesia and then subjected to necropsy. Group 1

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The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research. Further reproduction is authorized to satisfy needs of the U. S. Government.

Animals were exposed in 1961 by the Atlantic Research Corporation under ARPA sponsored contract AF 33(616)6623.

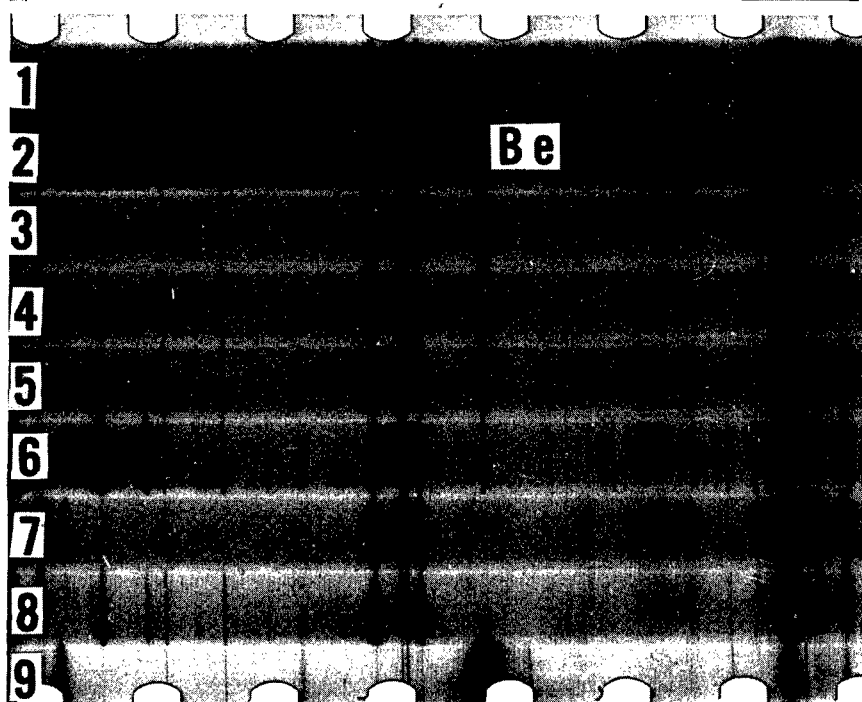
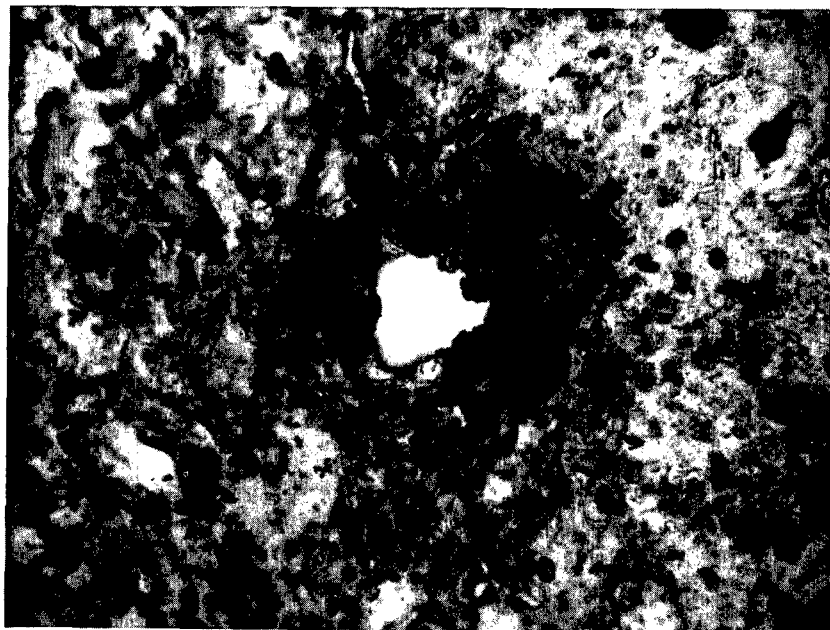


FIG. 1 (upper). Crater resulting from the incineration of a beryllium granuloma in a section of lung tissue $50\ \mu$ in thickness. Unstained. $\times 128$.

FIG. 2 (lower). Illustration of a spectrograph record ($\times 3$) showing the first order 3030 to 3031 Å doublet of beryllium (to the left of *Be*) in spectrums 2 through 6 and in the reference spectrum 9. Spectrums 1, 7, and 8 show no evidence of beryllium.

animals and both control dogs were sacrificed 30 months after exposure, whereas Group 2 animals were sacrificed 36 months after exposure.

Detailed necropsies were performed and tissues were preserved in citrated 10 per cent formalin for subsequent microscopic examination. Tissue blocks from each dog were

processed in the routine manner and embedded in Paraplast* embedding medium. Sections were cut 5 μ in thickness and were stained with hematoxylin and eosin. Pulmonary tissues selected for microemission spectrographic analysis (50 μ in thickness) were prepared from the same blocks and mounted on glass slides.

Microemission spectrographic analyses were performed with a laser microprobe.† Pulmonary granulomas and nongranulomatous pulmonary tissue, as well as the glass slides on which the specimens had been mounted, were analyzed for the presence of beryllium. Material for analysis was positioned in the microscopic field of the instrument, and the condensers and laser were energized. The laser beam was discharged through the microscopic optics, which caused incineration of a small zone of embedded tissue or glass. Figure 1 illustrates a crater formed in embedded lung tissue by the intense heat of the laser pulse. The cloud of vaporized tissue rising from the crater passed between a pair of carbon electrodes. A high voltage electric arc passed between the electrodes and through the vapor cloud 3 msec. after the laser discharge. The atoms from the tissue, ionized by the thermal energy provided by the laser beam and the electrical discharge, emitted a characteristic light spectrum that was recorded on photographic emulsion film in a classic 1.5-m. Wadsworth spectrograph. Optimal conditions for demonstration of beryllium involved an effective slit height of 2.0 mm., a slit width of 150 μ , and a focusing optic of 3 cm. The 35-mm. film (type 103-0) was developed for 3 min. before being placed in the fixative solution.

A mercury spectrum was utilized to orient the spectrums in the electromagnetic spectrum; a 2.5- by 2.5- by 0.2-cm. plate containing 99 per cent beryllium metal was used to provide a reference spectrum. By adjustment of the film holder, recording of 6 to 9 consecutive observations as parallel spectrums on 1 film was possible (Fig. 2).

* Scientific Products, Evanston, Illinois.

† Product of the Jarrell-Ash Company, Waltham, Massachusetts.

TABLE 1
SUMMARY OF RESULTS OF EXPOSURES OF DOGS
TO BERYLLIUM-CONTAINING DUSTS

	Group 1, Beryllium Oxide		Group 2, Halogenated Beryllium
	6 exposed	2 control	2 exposed
Beginning weight	9.1 kg.	9.9 kg.	9.4 kg.
Weight change 7 days post-exposure	-0.26 kg.	Normal gain	-1.5 kg.
Pulmonary granulomas	2	0	2
Beryllium in granulomas	2	0	2

RESULTS

Clinical Findings

The dogs were anorectic and lethargic for approximately 24 hr. after exposure to the beryllium-containing dusts. Thereafter, they appeared clinically healthy. Group 1 animals showed an average weight loss, 7 days after exposure, of 0.26 kg., whereas all Group 2 animals lost 1.5 kg. over the same period of time (Table 1). The time required to return to the original weights varied from a few days to 6 months. The control dogs continued to gain weight at a normal rate. At the time of necropsy, all dogs seemed to be in excellent physical condition and showed no clinical signs of disease. A detailed necropsy was performed on each animal, and no significant gross lesions were observed.

Histopathologic Findings

Histopathologic examination revealed characteristic beryllium granulomas in 4 dogs, 2 from Group 1 and both dogs in Group 2. Multiple, discrete, focal granulomas were scattered throughout the lungs in all 4 dogs (Fig. 3). The dog most severely affected was from Group 1, and the granulomas measured up to 500 μ in diameter, with occasional lesions exceeding 1 mm. The smaller granulomas observed in this animal consisted of a few centrally located macrophages containing small black particles superimposed on a yellow cytoplasm. The cytoplasm also

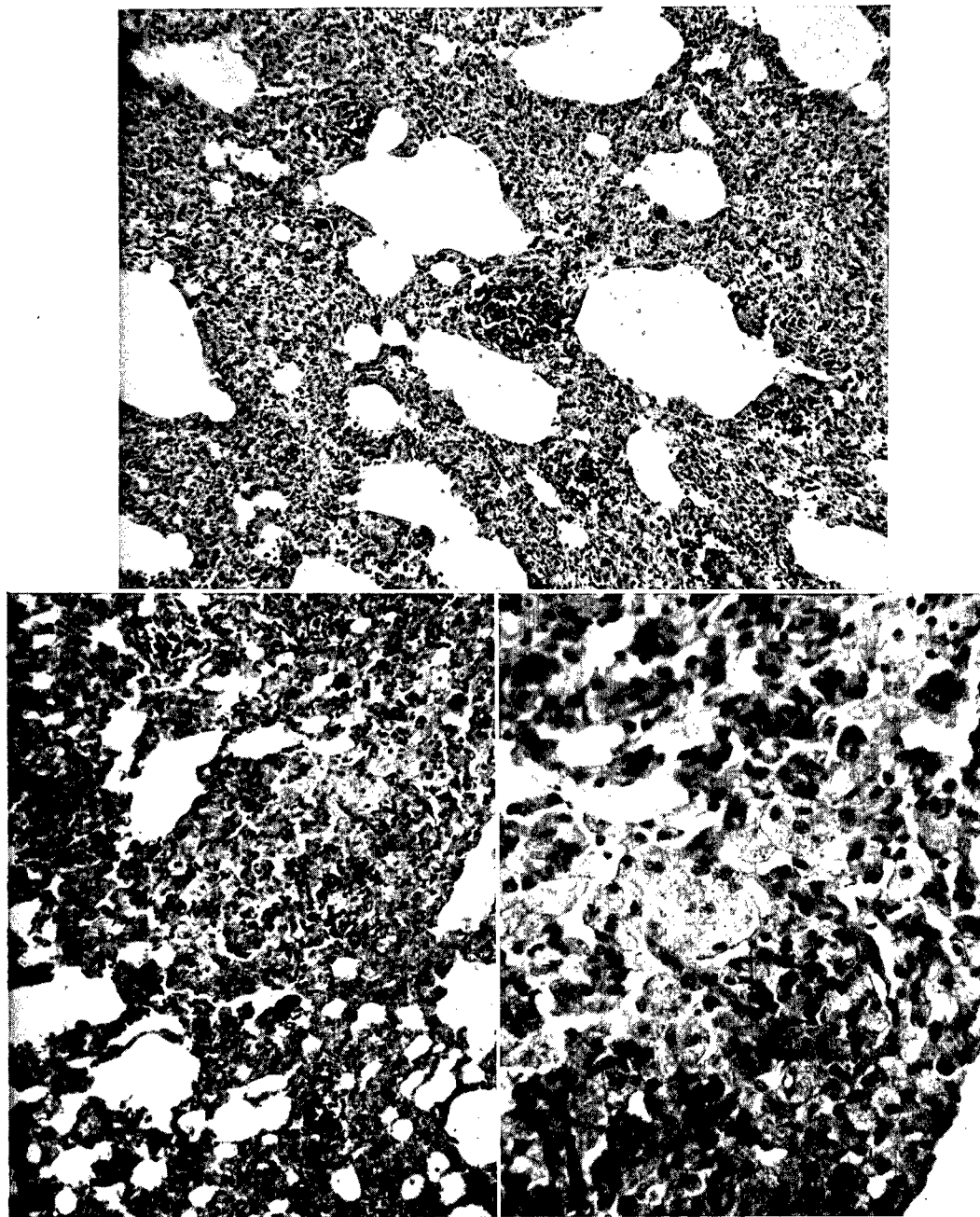


FIG. 3 (upper). Small multiple discrete beryllium granulomas in the lung of a dog exposed to a dust containing halogenated beryllium. Hematoxylin and eosin. $\times 70$.

FIG. 4 (lower left). Large beryllium granuloma composed of many large macrophages in the lungs of a dog exposed to a dust containing beryllium oxide. Hematoxylin and eosin. $\times 128$.

FIG. 5 (lower right). Beryllium granuloma in the lung of a dog exposed to dust containing beryllium oxide, showing large macrophages containing yellow pigment, but no dark particulate material. Hematoxylin and eosin. $\times 320$.

contained some small particles that had a slight degree of birefringence. Most of the particulate matter seemed to be intracellular in nature, although some particles were extracellular. Surrounding the macrophages was a mixture of both small and intermediate-sized lymphocytes and occasional plasma cells that tended to be located in the periphery of the granuloma. Larger reticuloendothelial (RE) cells were scattered throughout.

The larger granulomas in this dog were composed principally of large mononuclear macrophages with pale yellow-tinged cytoplasm (Figs. 4 and 5). Some of these cells also contained dark particulate material. Lymphocytes tended to form clusters in the interstices between these particulate-laden groups of macrophages. An occasional macrophage had a definite light yellow intracytoplasmic globule of material of poorly defined outline. This yellow globular material was observed in unstained sections, as well as in those stained with von Kossa's, Prussian blue, Masson's trichrome, and periodic acid-Schiff stains. Large nonphagocytic reticuloendothelial (RE) cells were scattered throughout these larger granulomas. There was no apparent association of the granulomas with the larger air passages, as the distribution of the granulomas was quite diffuse. The lesions in the lung of the other dog of Group 1 and in both dogs of Group 2 were smaller, but similar to the smaller granulomas described above. Excess collagen formation was not observed in the lungs of any of the 4 animals with pulmonary granulomas.

The bronchial lymph nodes contained a considerable quantity of brown-black pigmented material. The germinal centers of the lymph nodes were considerably enlarged and pale. The medullary sinuses were dilated and contained large numbers of RE cells with an abundance of eosinophilic cytoplasm. The total number of small lymphocytes in the nodes was significantly diminished.

Replicate sections of the lungs were incinerated in a high temperature oven. A dull birefringence was observed in the same zones where the previously described pigment and

birefringence had been noted in the sections stained with hematoxylin and eosin.

Chemical Findings

Results of the microemission spectrographic analyses of lung tissue from the affected dogs revealed that beryllium was detected only in the granulomas containing the yellow material. Beryllium was not detected in adjacent nongranulomatous lung tissue. There were some areas in the lung tissue that contained the black particulate material with no yellow pigment; these areas did not seem to contain beryllium. No beryllium was found in the 2 control dogs, nor was any found in parasitic granulomas from the lungs of other dogs that had not been exposed to the beryllium-containing dusts. Neither the glass slides *per se* nor Paraplast showed any evidence of the presence of detectable beryllium.

DISCUSSION

Beryllium has been previously demonstrated in tissues by various methods, including conventional spectrographic analysis, whereby the histologic integrity and structural relations have been destroyed in the analytic process.^{1-4, 6, 7, 9} We have, for the first time, we believe, identified beryllium within the specific histologic structures of pulmonary granulomas found in the lungs of dogs that had been experimentally exposed to dusts of beryllium compounds. The determinations of beryllium content in this study were not quantitative. The technic is being refined to allow at least semiquantitative estimates and to define the lower limits of detectability.

The dust used in the Group 1 exposures contained both beryllium oxide and free metallic beryllium. Precise analytic chemical data are not available for the beryllium products used in the Group 2 exposures.

The relatively low incidence of disease in human populations similarly subject to beryllium exposure has been enigmatic throughout all studies of beryllium disease. The fact that only 2 of 6 animals in Group 1 developed characteristic pulmonary granulomas further illustrates the problem of the apparent individual response to this metal, or to its salts, or to both.

Only 2 dogs were exposed to the beryllium dust containing halogens, but both developed the disease. Although the primary purpose of this investigation was to demonstrate the presence of beryllium in pulmonary tissue, it is interesting to speculate on the possibility that the halogen causes a certain degree of pulmonary irritation which may be requisite for the inclusion of beryllium and subsequent development of granulomas. The occurrence of granulomas in both dogs exposed to this particular compound, although not statistically significant, does support the theoretical consideration of extrinsic factors as possible contributors to the establishment of the disease.

The failure to detect beryllium in those areas of the lung containing only the brown-black particulate material indicates either that only the yellow material contained beryllium, or that the amount of beryllium contained in the dark material was below the limits of detection of the method used. The dusts containing beryllium to which the dogs were exposed also contained particles of carbon, which would appear microscopically similar to the dark particulate matter described.

The absolute demonstration of beryllium in pulmonary granulomas resulting from exposure to dusts containing beryllium is decisive in the diagnosis of beryllium disease and in the elucidation of its pathogenesis. The microscopic diagnosis of the acute disease may be more difficult, as there are no microscopic features which define its etiology.¹¹ The granulomatous pneumonitis of chronic human beryllium disease also may be difficult to distinguish from sarcoidosis or other diseases merely by the microscopic examination of biopsy material.^{6, 10} However, histopathologic diagnosis can now be strongly supported by microemission spectrographic analysis of affected tissue and suspect lesions. This technic would seem to be of significant assistance to the pathologist, despite the fact that beryllium may occasionally be detected in the lungs of persons with no known exposure to beryllium.^{2, 4} Significant amounts of beryllium have been recovered from the lungs of people without clinical beryllium disease, so that a diagnosis

can not be made solely on the demonstration of beryllium in pulmonary tissue.⁶

Since the time when the clinical disease was first associated with exposure to dusts containing beryllium, there has been no actual proof that the metal was present in the granuloma itself, even though its presence has been assumed. This tacit assumption has now been proved valid by the technic described. A valuable tool has been provided for further experimental investigations and for the purpose of confirming the diagnosis of beryllium disease in previously suspected cases.

Beryllium disease in humans has been diagnosed even when beryllium has not been demonstrated by spectrographic analysis of lung tissue obtained by biopsy, and when diagnosis has been made on the basis of clinical symptoms, history of exposure, clinical tests, and histopathologic observations. In general, however, good correlation has been reported between diagnosed cases of beryllium disease and recovery of beryllium from postmortem lung tissues. A major problem in previous technics for analysis of beryllium content has been the fact that quantities of lung tissue greater than those usually obtainable by biopsy were necessary for the identification of beryllium. Demonstration of a quantity of beryllium amounting to less than 1 μg . in pulmonary tissues of such cases may now be possible. More refined investigations of the pathogenesis of beryllium disease may determine whether the continued presence of beryllium is necessary for the progressive development of granulomas, or if a reaction similar to an autoimmune response mechanism is triggered, thereby eliminating the necessity for the continued presence of the original inciting agent.

SUMMARY

Chronic beryllium disease was induced experimentally in dogs, and the presence of beryllium was demonstrated in specific histologic structures (pulmonary granulomas) by means of a laser microprobe and emission spectroscopy. The ability to detect minute amounts of beryllium in tissue sections of necropsy and biopsy material

can be a significant aid in the diagnosis of both acute and chronic forms of beryllium disease. The detection of beryllium in histologic structures represents an important advance in the study of the pathogenesis of this disease.

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13. ABSTRACT Chronic beryllium disease was induced experimentally in dogs, and the presence of beryllium was demonstrated in specific histologic structures (pulmonary granulomas) by means of a laser microprobe and emission spectroscopy. The ability to detect minute amounts of beryllium in tissue sections of necropsy and biopsy material can be a significant aid in the diagnosis of both acute and chronic forms of beryllium disease. The detection of beryllium in histologic structures represents an important advance in the study of the pathogenesis of this disease.		

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