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INVESTIGATION ON THE PULMONARY EFFECTS OF INTERMETALLIC BERYLLIUM COMPOUNDS

Final Report

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ABSTRACT

The purpose of the contract was to investigate the pulmonary response to the exposure to tantalum and niobium beryllide, and a copper beryllium alloy. The findings were compared to beryllium metal as positive control. 2.5 or 0.5 mg as beryllium were given to rats by intratracheal intubation. At 30, 60, and 90 days after exposure the response was similar with each material. There was inflammatory infiltrate by lymphocytes, macrophage accumulation, and beginning fibrosis of the terminal bronchioles. Epithelial hyperplasia occurred at, or after, 90 days. Niobium beryllide had a unique granulomatous lesion which was similar to human berylliosis. After 15 months, 8 squamous cell carcinomas and 1 adenocarcinoma were found in rats exposed to beryllium metal. No neoplasms were seen with the other materials.

Solubility studies in saline and serum were conducted with the same materials. Beryllium metal and tantalum beryllide were dissolved up to 0.2 and 0.13 micrograms per milliliter in saline, respectively. The final concentration in serum was 0.23 and 0.12 micrograms per milliliter. Niobium beryllide had a significantly lower solubility, namely, 0.04 in saline and 0.06 micrograms per milliliter in serum. The copper alloy was even lower at 0.02 in saline and 0.01 micrograms per milliliter in serum.

The objectives of the investigation were to define the pulmonary response to the intratracheal application of intermetallic beryllium compounds. The endpoints of measurement were the morphologic alterations observed after serial sacrifice during the first three months after exposure and the occurrence of pulmonary neoplasms at 18 to 24 months after exposure.

The respiratory bronchioles are the sites of early pulmonary response. The toxicity of beryllium metal after inhalation is well defined. The pattern of response is characterized by infiltration of mast cells and lymphocytes, aggregation of macrophages, followed by interstitial fibrosis and changes in the epithelial lining of the terminal bronchiole and respiratory bronchiole. The latter are manifested by hyperplasia of the cuboidal and columnar epithelium with subsequent metaplasia to squamous cell epithelium. After this stage the development of neoplasms is observed. These pathologic alterations are in their occurrence dependent on the chemical and physical form of the beryllium compounds. The rat has been used extensively as the experimental animal. Pulmonary lesions caused by beryllium are better defined in this species than in others. Therefore, it was the animal of choice for this project.

Since the response of any tissue to a toxic agent depends, besides its absorption, to a large extent on its solubility, a comparison was made between the solubility of metallic beryllium and the intermetallic compounds. These were tantalum and niobium beryllide and a 4% copper beryllium alloy. The studies were conducted with physiological saline and serum.

The research was delayed for about six months. One reason for this was that the beryllium compounds were not received before three months had passed from the starting point of the contract. Another reason was that the rats exposed developed a severe pulmonary infection. It was evident that the mortality would be so great that no sufficient number of animals would survive for 18 to 24 months to evaluate the carcinogenicity of the compounds. Therefore, the experiment was started over with new animals. This delayed the initiation of the research into the late fall of the year of the contract.

EXPERIMENTAL DESIGN

Material and Methods:

The three compounds which were tested were: $NbBe_{12}$, $TaBe_{12}$, and $CuBe_2$. The compounds were supplied as particles of respirable size by the National Institute of Occupational Safety and Health. The beryllium metal which served as the positive control came from the same source.

The compounds suspended in physiological saline were administered to Sprague Dawley male rats by intratracheal intubation. The design followed is given in Table 1. The table summarizes the number of animals used for each concentration and compound and the schedule of sacrifices. The dose given to the rats was calculated to be either 2.5 or 0.5 mg as beryllium.

At the time of sacrifice the lungs were removed *in toto*. Under a pressure of 20 mm of water neutral formalin was applied into the trachea. After this application the lungs were immersed into the same fixative. Intrathoracic lymph nodes and the lymph nodes of the neck were excised and fixed in neutral formalin. After fixation several

blocks from the lungs were embedded in paraffin and cut at 6 - 8 microns. For microscopic examination the sections were stained with hematoxylin and eosin. The histologic examination was done without the knowledge of the applied compound and concentration. The findings were recorded and afterwards tabulated in compliance with the experimental design.

TABLE 1

Compound	Dose in mg as Be	Number of Rats Injected	Number of Rats Sacrificed Days Following Injection			
			7	30	90	540
Be metal	2.5	55	5	5	10	30
Be metal	0.5	55	5	5	10	30
$CuBe_2$	2.5	55	5	5	10	30
$CuBe_2$	0.5	55	5	5	10	30
$TaBe_{12}$	2.5	55	5	5	10	30
$TaBe_{12}$	0.5	55	5	5	10	30
$NbBe_{12}$	2.5	55	5	5	10	30
$NbBe_{12}$	0.5	55	5	5	10	30
Saline	1 cc	55	5	5	10	30
TOTALS		495	45	45	90	270

The solubility studies were conducted with physiological saline and serum. Of each compound 2.5 mg as beryllium were added to 10 ml of saline or human serum. Polyethylene tubes were used to prevent any possible interaction of beryllium with glass containers. The saline suspensions were kept at 25°C and stirred daily by an electric stirrer. For the first two weeks one sample of each compound was analyzed for beryllium every other day; after that monthly analyses were done. The tubes were centrifuged and 5 ml of the saline were taken for the determination of beryllium. The procedure was discontinued after a plateau was reached. The serum suspensions were handled in a similar way. Before 2.5 mg as beryllium of each compound were added, the serum was refrigerated for several days and any clots that had developed were removed by filtering through a paper filter. Samples were kept under refrigeration and stirred periodically. Preparation for chemical analyses were done in the same manner as described for the saline solutions. After 6 weeks a plateau was reached.

The methods used for the determination of beryllium were those methods used routinely by the Analytical Section of our Department.

All aqueous solutions were screened by Atomic Absorption Spectrophotometry[1] to determine the concentration necessary to obtain the best results.

The samples were wet ashed with nitric and sulfuric acids as described by Hiser, R.A. et al[2]. The resulting ash was dissolved in a minimum amount of solution and analyzed by Atomic Absorption Spectrophotometry[1], which has a detection capability of approximately 0.003 micrograms per ml of solution. Samples

containing lower amounts of beryllium were analyzed by the Spectrographic Method of Cholak, J. et al[3], which has a detection of 0.003 micrograms per sample.

RESULTS

The major objectives of this research were to compare, in a short-term experiment, the early pulmonary response to beryllium metal, tantalum and niobium beryllide, and a copper beryllium alloy. Secondly, in a chronic study the carcinogenicity of the different materials was to be ascertained. The application was by intratracheal intubation of either 2.5 or 0.5 mg as beryllium.

The intubation was staggered over a period of 3 months to accomplish a better schedule for sacrifice at the termination of the long-term experiment. An equal number of rats from each shipment was intubated with each compound and dosage. The animals from the first shipment (180) had to be destroyed because of severe murine pneumonia. This infection would have had a marked effect on the outcome of the experiment. The lungs of this group of rats were examined histologically. The severity of the pneumonia was more pronounced in those animals that had received the metallic beryllium or the tantalum beryllide. Most of these rats had died within 10 days after the intubation. The niobium beryllide had a moderately lesser effect, whereas, the least severe incidence was present after the application of the copper-beryllium alloy.

After all long-term animals were treated, the short-term experiments were undertaken. These rats were exposed for 30, 60, or 90 days. After 30 days of exposure, the pulmonary response differed among the experimental groups.

Beryllium caused a greater response in the number of pulmonary macrophages in the alveoli. These cells contained dark black-gray granules. The terminal and respiratory bronchioles exhibited slight to moderate infiltration by lymphocytes. No fibrosis or epithelial hyperplasia was seen at 30 days of sacrifice. At 60 and 90 days the accumulation of macrophages had decreased. Fibrosis of the terminal bronchioles was moderate.

After tantalum beryllide, there was fibrosis of the respiratory bronchioles of a slight degree at 30 days. This was increased after 60 and 90 days. Also, slight hyperplasia of the epithelium in the terminal bronchioles was present. This was not seen in any of the other groups. The response by pulmonary macrophages was minimal.

The niobium and copper beryllide exhibited an infiltration by lymphocytes in the respiratory bronchioles. There was a moderate number of pulmonary macrophages. These contained gray granular material. At 90 days sacrifice, some macrophages with the granular material were still present.

The rats on the long-term experiment were treated with penicillin and tetracycline prophylactically two days before and ten days after the intratracheal intubation of the materials. During the course of 18 months the morbidity and mortality was greater than expected. In Table 2 the mortality data are summarized.

According to the experimental design (Table 1) 5 rats had been added as a precaution in case the mortality would be high. Unfortunately, the number of survivors was lower than the anticipated one, namely, 20 rats per group at 18 months. Only two animals in the 2.5 mg beryllium metal group exhibited a pulmonary carcinoma in the

ones that died 16 and 17 months after exposure. This was the reason why the rats were maintained beyond the 18 months. In our experience with other pulmonary carcinogenicity studies, neoplasms in the lung usually become manifested after that period of time.

TABLE 2

Mortality at 16 and 18 Months of Long-Term Experiment

Compound	Survivors 16 Months	Survivors 18 Months	Survivors 24 Months
Be metal 2.5 mg	15	12	6
Be metal 0.5 mg	19	15	9
$CuBe_2$ 2.5 mg	20	16	10
$CuBe_2$ 0.5 mg	20	17	10
$TaBe_{12}$ 2.5 mg	12	10	5
$TaBe_{12}$ 0.5 mg	14	11	6
$NbBe_{12}$ 2.5 mg	18	16	8
$NbBe_{12}$ 0.5 mg	21	17	11
Saline 1.0 ml	23	19	12

The cause of death in most animals was murine pneumonia. A small number had either a severe kidney infection or enteritis.

A complete autopsy was performed on all animals that died or were sacrificed at the termination of the experiment. From each lung 8 to 10 blocks of tissue were taken and examined histologically.

The saline control group had normally structured lungs except for pneumonia. It was the typical murine pneumonia with bronchiectasis and abscess formation.

An early morphologic response to the beryllium metal is depicted in Figure 1. This rat was sacrificed at 90 days. The wall of the terminal bronchiole is thickened and densely infiltrated by lymphocytes. There is hyperplasia of the epithelium of the bronchiole. Fine black particles are still present, either suspended in some mucus or contained in macrophages. At a later stage (14 months) focal fibrotic scars are present. A small number of lymphocytes can be seen (Fig. 2).

The tantalum beryllide rats exhibited similar pulmonary responses as the beryllium metal ones. Dense infiltration by lymphocytes and epithelial hyperplasia of the terminal bronchioles in earlier stages and focal fibrosis after 10 months were seen. An occasional animal showed granulomas scattered throughout the lungs, as demonstrated Figure 3. The center consists of large macrophages, whereas, the periphery is composed of accumulations of lymphocytes. Multinucleated giant cells were not present. A moderate degree of pulmonary emphysema was seen (compare Fig. 3 with Fig. 2).

The lungs of rats exposed to niobium beryllide showed a unique response. Many granulomas were present, either single or in conglomerates. Figures 4 and 5 are demonstrating this lesion. The granulomas are loosely structured. The centers are composed of multinucleated giant cells, some of which have a bizarre syncytial shape. The periphery is made up of lymphocytes and a small number of histiocytes. This granuloma compares well with those seen in human berylliosis. Later stages show fibrosis of the granulomas (Fig. 6). Occasionally a multinucleated giant cell is still present. The lungs with the fibrotic lesion also have a moderate degree of centrilobular emphysema.

Figures 7, 8, and 9 depict the pulmonary reaction to the copper beryllium alloy. The early response, up to 4 months, consists of accumulations of macrophages ladened with minute particles and lymphocytes (Fig. 7). Fibrosis of bronchioles and alveolar walls develop later. Pulmonary macrophages are still prominent, but foreign granular material is absent. Lymphocytic infiltration persists at a slight degree (Fig. 8). Even granulomatous lesions were not seen; focal nodular fibrosis occurred occasionally (Fig. 9). These fibrotic nodules have a circular lamellar structure. It is a slightly loose arrangement with a small number of lymphocytes.

The beryllium metal was the only material that caused pulmonary neoplasms. The rats treated with 2.5 mg of the metal had 9 squamous cell carcinomas, whereas the 0.5 mg group had 1 squamous and 1 adenocarcinoma. The timely occurrence of these tumors is summarized in Table 3.

TABLE 3

Timely Occurrence of Pulmonary Neoplasms in Beryllium Metal Rats

Dose of Beryllium Metal	Month										
	14	15	16	17	18	19	20	21	22	23	24
2.5 mg			1	1	2		1	1	1		1
0.5 mg							1			1*	

* Adenocarcinoma.
All other tumors were squamous cell carcinomas.

Representative pictures of the neoplasms are presented in Figures 10, 11, and 12. Figures 10 and 11 show a squamous cell carcinoma. The

invasiveness is well demonstrated. There is a moderate amount of supporting fibrous tissue in the center of the tumor. At the periphery it is extending into the alveolar spaces (Fig. 11) with destruction of the pulmonary tissue. The adenocarcinoma was basically composed of columnar cells (Fig. 12). The cells are arranged in irregular glandular patterns. In the center of the picture the bronchus from which the tumor originated can be seen. The entire wall of this bronchus is replaced by neoplastic tissue.

After the short-term experiment (up to 90 days) was completed, the solubility study was conducted. The saline suspension reached a plateau after the fourth determination; however, the analysis was continued according to the schedule outlined under "Methods". The concentration of beryllium in the serum reached the plateau after two determinations. In Table 4 the final concentration achieved in saline and serum is given and expressed in micrograms per milliliter.

There were no significant differences between the saline and serum as solvents. The solubility of each compound was of low degree. Considering that the detection limit of the analytical method is 0.03 micrograms per milliliter of solution, the values for NiB_{12} and the copper-beryllium alloy are borderline. They are significantly lower than for beryllium metal and the tantalum beryllide.

TABLE 4

Concentration of Beryllium Achieved in Saline and Serum

Compound	Solvent	Beryllium micrograms/milliliter*
Be metal	saline	0.2
$TaBe_{12}$	saline	0.13
$NiBe_{12}$	saline	0.04
$CuBe_2$	saline	0.02
Be metal	serum	0.23
$TaBe_{12}$	serum	0.12
$NiBe_{12}$	serum	0.06
$CuBe_2$	serum	0.01

* By atomic absorption. Detection capability 0.03 micrograms per milliliter.

COMMENTS

The investigation revealed significant differences in the pulmonary response to the intubation of the four materials. The beryllium metal and the tantalum beryllide seem to be more toxic than the other two compounds. The type of lesion was as expected in Be_2, $TaBe_{12}$, and $CuBe_2$. The stages of inflammation, epithelial hyperplasia, and fibrosis were verified. The granulomatous lesion after exposure to $NiBe_{12}$ was unique for this compound and surprisingly similar to human berylliosis. No explanation for this can be given.

The difference in carcinogenicity was not foreseen. Only the pure metal caused development of neoplasms. One would have expected that the copper beryllium alloy would have acted similarly. The fact that there was no tumor development might be explained by physico-chemical properties of the alloy, which would be better known to experts in metallurgy. A contributing factor might be the very low solubility of the alloy. In this regard one should consider that the solubility studies were conducted in a static system. In the lung the molecules in solution are taken up by cells and tissues. Therefore, more material can go into solution. For this reason, determination of absorption and retention of the compounds by the lung would be of greater value.

All materials had a significant toxicity. Because of this fact alone, the handling of the beryllides and the alloy should be governed by the existing regulations, namely, the same as for beryllium metal. Even though they exhibited no carcinogenic potential, this does not seem enough to relax the regulations.

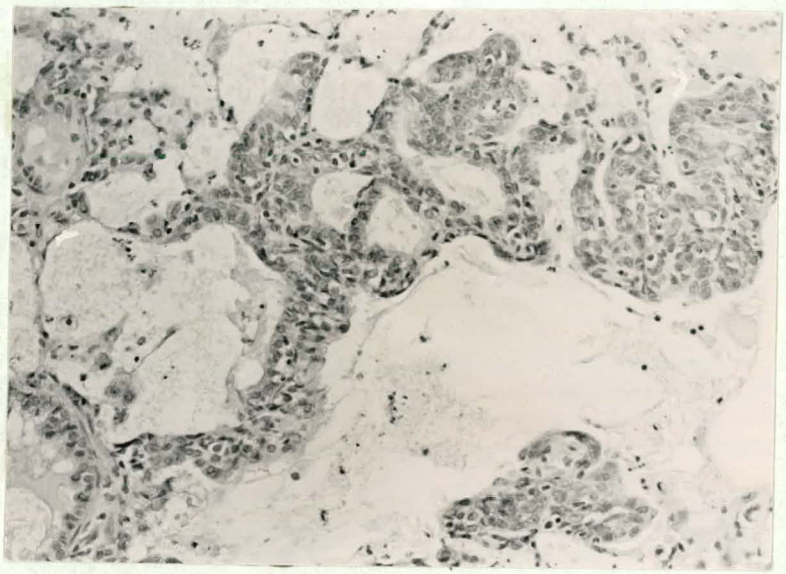

FIGURE 1: Terminal bronchiole with thickened wall by infiltrate of lymphocytes and epithelial hyperplasia. Adjacent alveolar walls are also involved. A few pulmonary macrophages are present. Fine particular material can be seen suspended in mucus material.
Rat exposed to beryllium metal after 90 days.
Original magnification 250x. Hematoxylin and eosin.

FIGURE 2: Focal fibrosis of terminal bronchioles and alveolar walls with lymphocytic infiltrate.
Rat exposed to beryllium metal after 14 months.
Original magnification 250x. Hematoxylin and eosin.

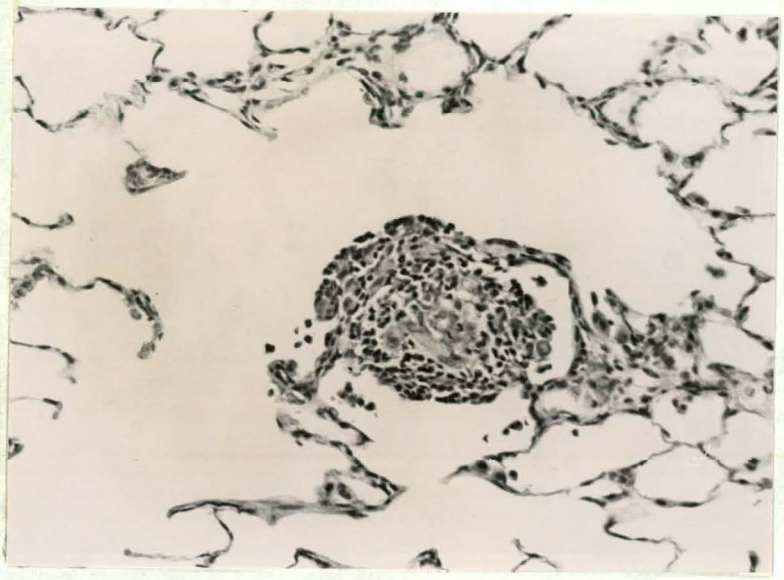

FIGURE 3: Granuloma in wall of bronchiole. Center of it is composed of macrophages, whereas the periphery consists of dense accumulation of lymphocytes. Multinucleated giant cells are absent. Moderate centrilobular emphysema is present. Compare with Figure 2. Both are of the same magnification. Rat exposed to tantalum beryllide after 10 months. Original magnification 250x. Hematoxylin and eosin.

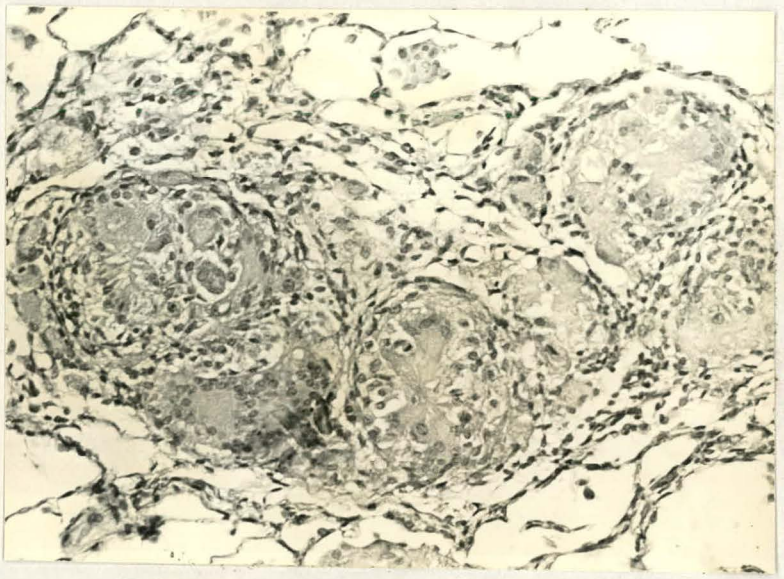

FIGURE 4: Conglomerate of granulomas in lung of rat exposed to niobium beryllide at 12 months. The center of the granulomas consists of multinucleated giant cells. In the periphery are lymphocytes and histiocytes.

Original magnification 250x. Hematoxylin and eosin.

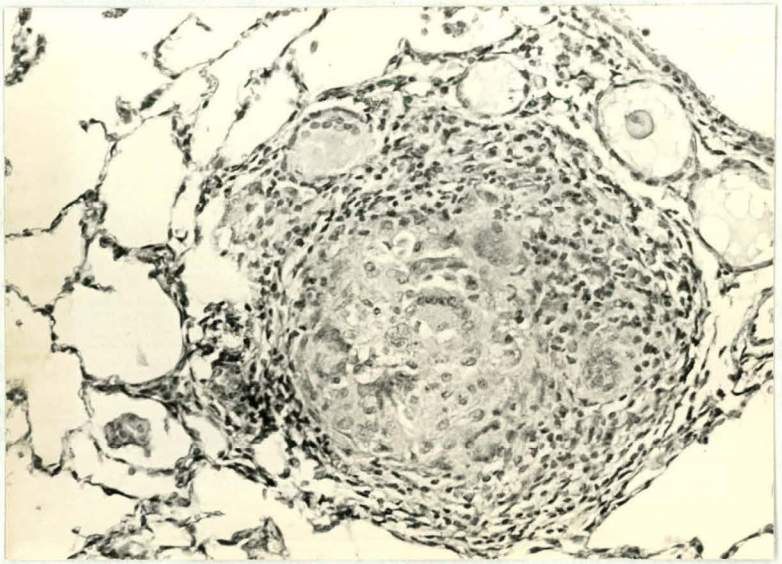

FIGURE 5: Higher magnification of a single granuloma showing its distinct cellular composition.
Rat exposed to niobium beryllide after 9 months.
Original magnification 450x. Hematoxylin and eosin.

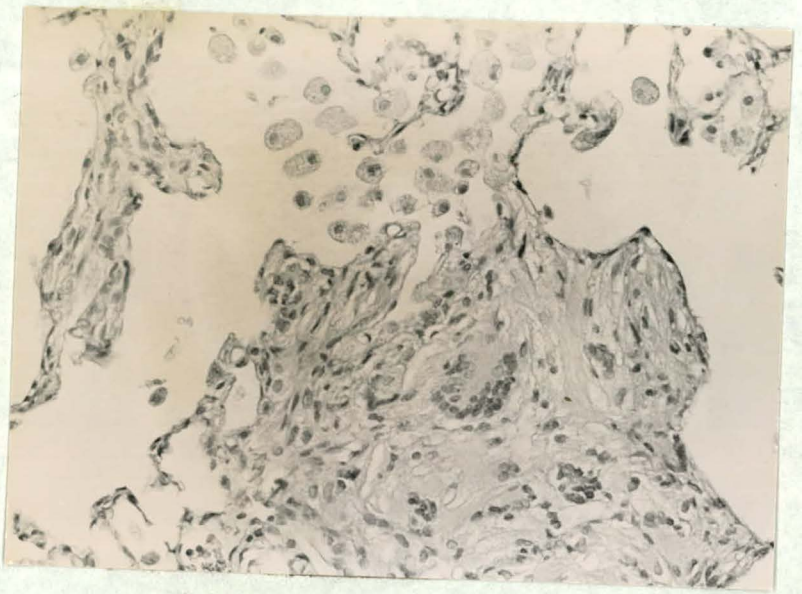

FIGURE 6: Fibrotic granuloma after 19 months of exposure to niobium beryllide. Giant cells are still present. Diffuse fibrosis can be seen in opposite bronchiolar wall. Small number of pulmonary macrophages can be recognized.

Original magnification 450x. Hematoxylin and eosin.

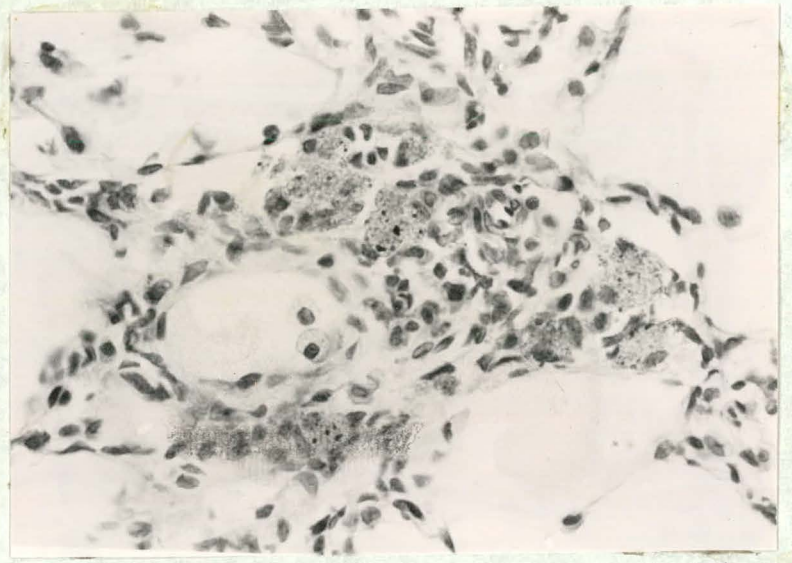

FIGURE 7: Lung of rat 4 months after exposure to copper beryllium alloy. There are large pulmonary macrophages containing fine particular material. Some lymphocytes and fibroblasts are present. Original magnification 250x. Hematoxylin and eosin.

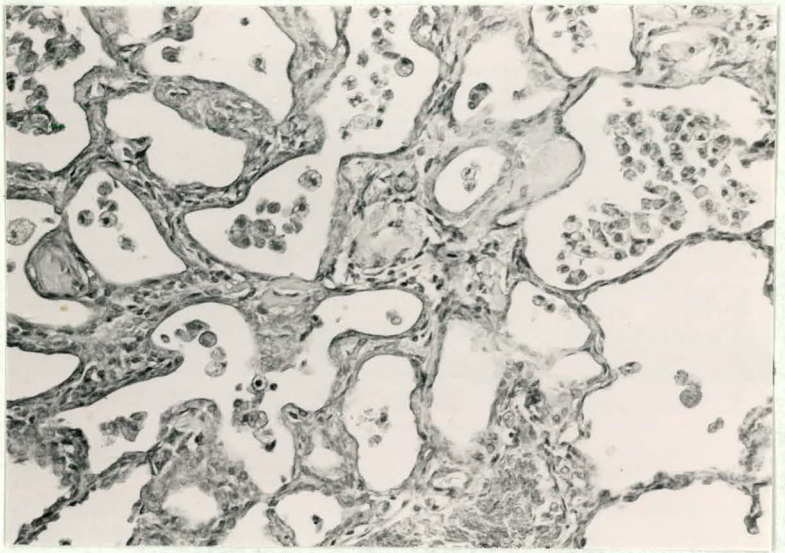

FIGURE 8: The lung shows diffuse fibrosis with slight infiltrate by lymphocytes. Pulmonary macrophages are present in small numbers.
Rat exposed to copper beryllide alloy after 18 months.
Original magnification 250x. Hematoxylin and eosin.

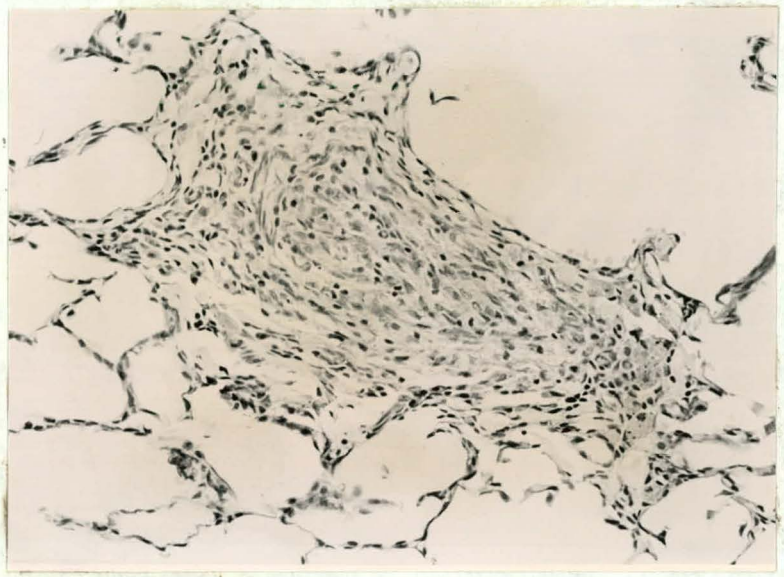

FIGURE 9: Focal fibrosis in lung of rat exposed to copper beryllium alloy after 11 months. The fibrotic nodule shows a circular laminar structure. Some lymphocytes are present.
Original magnification 250x. Hematoxylin and eosin.

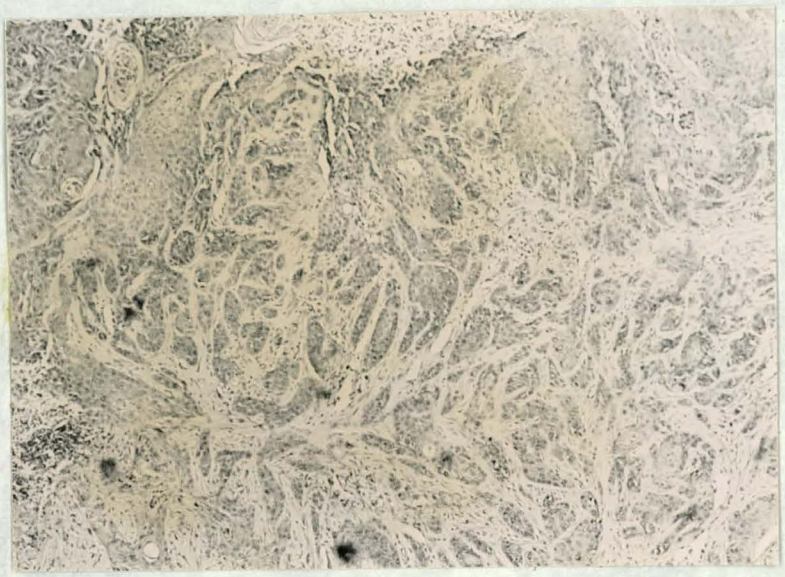

FIGURE 10: Squamous cell carcinoma in lung of rat exposed to beryllium metal at 18 months. The tumor is invasive and supported by fibrous tissue. Original magnification 50x. Hematoxylin and eosin.

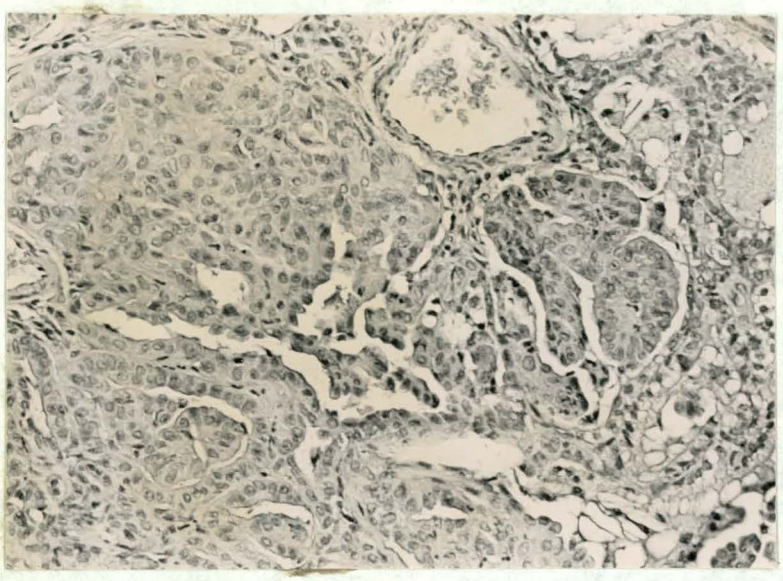

FIGURE 11: Higher magnification of the periphery of the squamous cell carcinoma of Figure 10. The cellular type of the neoplasm and its invasiveness are demonstrated.

Rat exposed to beryllium metal at 18 months.

Original magnification 250x. Hematoxylin and eosin.

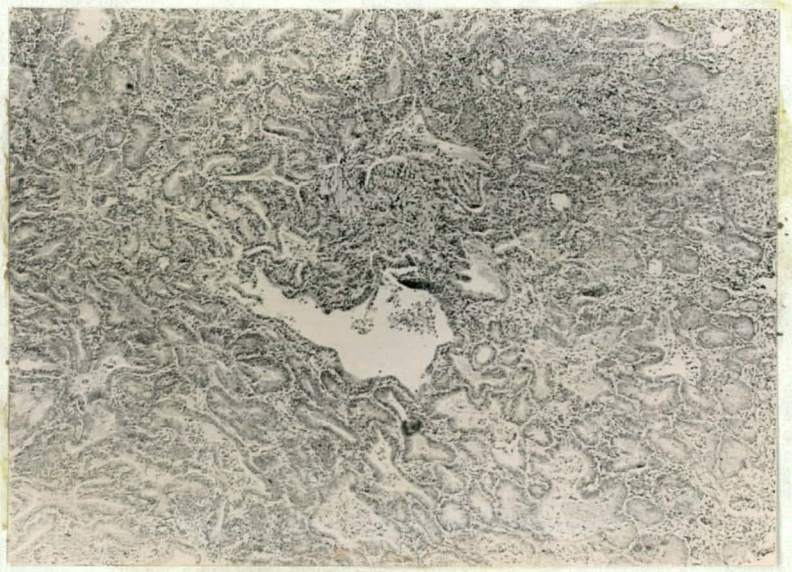

FIGURE 12: Adenocarcinoma in lung of rat exposed to beryllium metal at 23 months. The cell type is columnar to low columnar. The tumor originates from the centrally located bronchus. The cells are arranged in irregular acinar and glandular structures. Original magnification 50x. Hematoxylin and eosin.

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2. Hiser, R.A., Donaldson, H.M., Schwenzfeier, C.W.: A rapid analytical method for the determination of beryllium in air samples. Am. Ind. Hyg. Assoc. J. 20:241 (1959).

3. Cholak, J., Hubbard, D.M.: Beryllium analysis in biological and related materials. Am. Ind. Hyg. Assoc. J. 13:3 (1952).