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PLUTONIUM PARTICLE-INDUCED NEOPLASIA OF THE CANINE LUNG
II. HISTOPATHOLOGY AND CONCLUSIONS*

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INTRODUCTION

Previous papers have described the clinical and gross pathology in 37 beagle dogs that have succumbed following exposure to $^{239}\text{PuO}_2$ aerosols (Bair and Willard, 1962; Park et al, 1962; West and Bair, 1964; Clarke and Bair, 1964). This report describes the sequential developments leading to lung tumors in 5 of these animals during a 5 year post-inhalation period.

EXPERIMENTAL METHODS

Beagle dogs were given single 10-to-30 minute exposures to $^{239}\text{PuO}_2$ aerosols. Techniques and descriptions of the exposure apparatus have been reported previously (Bair and McClanahan, 1961). The count median particle diameter of the aerosols ranged from 0.1-to-0.5 μ . At sacrifice or spontaneous death, tissue samples for histopathologic study were fixed in 10 percent neutral buffered formalin. Routine hematoxylin and eosin preparations were made, supplemented with autoradiographs, corresponding to each H&E section, prepared on NTA emulsions.

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RESULTS

Immediately following particle exposure, there was a heavy, diffuse distribution of activity throughout the entire lung (fig. 1). By the seventh post-exposure day the conducting and respiratory lumina were devoid of activity; the remaining medium to large-sized particles were located mostly in the walls of the terminal bronchioles and the smaller particles in the alveolar septa. The residual particles, tending to adhere to the less mobile parts of the moist intra-bronchiolar and alveolar surfaces, had been engulfed by macrophages or had penetrated the cytoplasm of the alveolar epithelial cells to reach the connective tissue beneath the alveolar epithelium. During this period, other than a slight septal cell proliferation and a moderate loss of mature lymphocytes in the bronchial lymph nodes, the lungs and lymph nodes of these animals appeared normal.

At 14 days post-exposure, moderate alveolar septal cell proliferation ensued, accompanied by increased phagocytic pickup of the smaller particles. The larger particles continued to remain in the vicinity of the smooth muscle ring in the terminal bronchioles. With this interstitial concentration of the remaining particles, some of the small bronchioles at 50 to 60 days showed epithelial damage and a few foci of peribronchiolar fibrosis and lymphocytic and macrophagic infiltration (fig. 2).

By 65 days post-exposure, septal cell radioactivity appeared to decrease while alveolar macrophage activity increased. Particles located within the terminal bronchiole walls, however, did not seem

to vary in number or location with time. Septal and focal fibrosis became increasingly severe, resulting in a partial obliteration of the pulmonary architecture in some areas. The appearance of alveolar cell metaplasia was focal and moderate in amount, but was overshadowed by a mild to severe peribronchiolar squamous and bronchiolar-type metaplasia, the alveolar lining cells taking on the appearance of squamous or simple columnar epithelium (figs. 3 and 4). These metaplastic changes were especially prominent in the areas of deposition of radioactivity and fibrosis. Concurrent with these changes was the appearance of increasing amounts of activity in the region of the peribronchial and bronchiolar, and subpleural lymphatics, and in the bronchial lymph nodes. The bronchial lymph nodes showed an extensive loss of lymphocytes, small scattered foci of necrosis, moderate to severe fatty change, small islands of residual lymphatic tissue, and many macrophages containing hemosiderin pigment. The mediastinal nodes were unaffected.

At 124 through 384 days post-exposure, there was extensive squamous and alveolar cell metaplasia present in groups of alveoli around some of the bronchioles. Fibrous thickening of alveolar septa and foci of advanced dense fibrosis were prominent. Hypertrophy and hyperplasia of the lining epithelium could be seen in some intra-lobular bronchi. Pleural and sub-pleural fibrosis noted in some of these longer-lived animals was suggestive of the pleural scarring seen in X-irradiation pneumonitis. However, following heavy doses of X-irradiation, fibrinous pleural adhesions are common, a condition that was not seen in these animals. Instead, pleural scars were associated

with heavy depositions of radioactivity in the regions of the pleural lymphatics, resulting in fibrous condensation of the lung parenchyma in these areas. The bronchial lymph nodes showed large areas of lymphocytic depletion and replacement by reticulum cells, leaving only small residual lymphatic nodules.

By 855 days post-exposure, severe septal and peribronchiolar fibrosis, and alveolar cell metaplasia, were prominent. Hemosiderin-filled macrophages were present around the bronchioles in some of the more heavily fibrosed areas. There was a diffuse increase in alveolar macrophages, the appearance of a few giant cells in the alveoli, hyalinization of some of the alveolar septa, and a scattered infiltration of neutrophils around some of the terminal bronchioles. The bronchial lymph nodes showed a complete loss of lymphocytes with fibrous connective tissue replacement of the lymphatic tissue and fibrosis of the vessels. The mediastinal nodes, on the other hand, showed only a moderate loss of lymphocytes with concomittant fibrosis and an increase of reticulum cells.

Of the 10 animals surviving 29-to-56 months post-exposure, 5 evidenced bronchiolo-alveolar carcinomas. The tumors were peripheral or subpleural in location, and all were multicentric in origin, except in one dog where only one neoplasm in the right apical lobe was observed.

The lungs of these animals showed marked pleural, subpleural, parenchymal, and alveolar septal fibrosis and sclerosis. In addition, other large areas appeared to be undergoing consolidative processes, consisting of septal cell proliferation, alveolar macrophage increase, giant cell formation, hemorrhage, and varying degrees of fibroplastic

response. Scattered throughout, in association with some of these fibrosed and sclerotic areas, were numerous foci of alveolar and bronchiolar type metaplasia. Transition from metaplasia to neoplasia could be seen in many of these regions as pleomorphic cells with epithelioid or fusiform characteristics infiltrated the surrounding parenchyma. In some instances, the neoplastic cells formed the basis of frond-like or papillary projections (fig. 5), while in others a bronchiolar or glandular structure was apparent (fig. 6). Ciliation was not seen in any of the metaplastic or neoplastic cells.

In the areas of bronchiolar or gland-like pattern the cells often took on the appearance of squamous or glandular epithelium. Those areas with a papillary semblance, in contrast, have very anaplastic looking cells showing loss of polarity and nuclear displacement toward their luminal tips. Throughout the parenchyma of many of the lungs, small focal clumps of hyperchromatic, anaplastic cells could be seen, suggestive of nests of early neoplasia. In addition, some of the metaplastic foci showed gradational transition to a loose, streaming pattern of fusiform or spindle cells similar to those found in undifferentiated bronchiolar carcinoma (fig. 7). Mitotic figures, though not numerous, were present in all neoplasms.

Due to compression atelectasis of the surrounding lung parenchyma, there was a tendency toward encapsulation in some of these tumors; however, encapsulation in most instances was not complete and there was extension of the tumor cells into the surrounding tissue. In the larger, more anaplastic neoplasms, focal areas of necrosis and calcification of the necrotic debris could be seen. Although local

invasiveness was apparent in all of these tumors, metastases were found only in one animal. In this dog, metastatic foci from the primary lung tumors were seen in the middle and left bronchial lymph nodes (fig. 8). Extension to other tissues or organ systems, however, was not apparent.

The origin of these bronchiolo-alveolar tumors appeared to be from the bronchiolar and/or alveolar epithelium in all cases. In no instance could derivation be associated with bronchial epithelium or bronchial glands.

Perivascular and peribronchiolar fibrosis was generalized and severe. Many of the pulmonary vessels showed a marked thickening of their media, intimal proliferation, and in the case of the arteries, fracture and disorganization of the elastica interna, and an increase in and vacuolation of the subendothelial connective tissue in the tunica intima.

Terminal bronchioles in these damaged areas either manifested a denudation or bizarre squamous forms in their epithelial linings.

Serial autoradiographs of these lung sections showed moderate numbers of radioactive particles associated with the desmoplastic and metaplastic processes (fig.9). All of the neoplasms originated in peripheral lung areas of particle retention, fibrosis, and bronchiolo-alveolar metaplasia. The irradiated proliferating cells, comprising the metaplastic response to lung scarring, appeared to be the nidus of tumor formation.

The bronchial lymph nodes of these animals were composed of dense, sclerotic connective tissue and were practically devoid of lymphoid elements. Masses of hemosiderin and anthracotic particle

bearing macrophages were prominent in the central portions of the nodes. Serial autoradiographs of these sections showed a heavy, diffuse distribution of radioactivity throughout the scarred parenchyma (fig. 10). The mediastinal nodes, on the other hand, evidenced varying degrees of fibrosis and sclerosis, dependent on their radioactive particle content.

DISCUSSION

In the dog, the incidence of primary pulmonary neoplasms averages 0.2 percent in necropsy material (Nielsen and Horave, 1960; Sedlmeier and Dahme, 1958). Of these, bronchiolar adenocarcinomas constitute three-fourths of the tumors, followed by bronchial carcinomas and bronchial adenomas. Metastases are relatively infrequent with bronchiolar adenocarcinomas, compared to its counterpart in the human lung, and are frequent in bronchial carcinomas. Although the tumors found in this inhalation study have some characteristics similar to bronchiolar adenocarcinomas, certain differences preclude their inclusion in this category. Their peripheral location, apparent multicentric origin in relation to pulmonary fibrosis and scarring, and their cellular and organizational pleomorphism suggest rather that they are more analogous to pulmonary scar tumors or bronchiolo-alveolar tumors as described in man (Strauss et al, 1963; Kress and Allan, 1963).

Tumor production appeared to be dependent upon a combination of factors, among which were dose rate and total dose, degree of lung fibrosis, metaplastic response, and the spatial relationships between the radioactive particles and the metaplastic tissue at risk, in this

case the bronchiolar and/or alveolar epithelium.

Verification that radionuclides are pulmonary carcinogens has been accomplished experimentally in several other species of animals using various means of exposure; inhalation, intratracheal injection, and implantation of large sources in the lung (Temple, et al, 1960; Lisco, 1959; Abrams et al, 1946; Temple and Bair, 1960). Both alpha- and beta- emitting elements have been administered, principally in rodents. In these studies, as well as in the experiment under discussion, the neoplastic response suggested a general pattern of increased incidence with moderate doses, and diminished incidence with large doses of the radioactive material. This is entirely compatible with the carcinogenic influence of ionizing radiation at the former levels, and a possible sterilizing effect of the tumor bed of excessive amounts of radiation at the highest levels. However, the common bond of similarity between most of these experiments appears to lie in the fibroplastic and metaplastic response to the radiation insult. It has been shown that the deposition of fibrous tissue in the sequence of events in organizing pneumonitis is the stimulus for bronchiolar hyperplasia and squamous metaplasia. A similar response can be provoked by the deposition of radioactive particles in the lungs. It is not unreasonable to assume, therefore, that these abnormal tissue elements might serve as the nidus of tumor origin under the adjuvant influence of the radioactivity in the field.

The role of lung fibrosis as the antecedent or accompanying lesion in certain neoplasms of the human lung has also been recognized. Many articles have been published in the last century and early part

of this century associating fibrosing processes and malignancy of the lung with the uranium ore industry of Europe (Doll, 1958). A small but definite increase of cancer of the lung among uranium miners of the Colorado plateau has also been noted, although lung fibrosis did not seem to be as prominent in these cases as those of the European miners (Saccomanno et al, 1964). Lung cancer in association with pneumoconiosis has been noted in certain other occupations. In fact, most of the occupations in which a special risk of lung cancer has been established can also give rise to pneumoconiosis. The lung cancers that have occurred among asbestos workers have been almost uniformly associated with a severe form of pulmonary fibrosis. Chromitosis, a spotty fibrous thickening of the interstitial tissue, accompanies the development of lung cancer in chromate workers. In both of these preceding cases, the pulmonary fibrosis occurs in direct association with the specific irritant. In contrast to these observations, however, silicosis occurs commonly in some occupations in which the risk of lung cancer is normal or low, such as among coal miners. Therefore, it seems unlikely that the fibrotic reaction of the lung to inspired dust particles can be, in itself, a significant cause of cancer, but it may be an important contributing factor when a specific carcinogen, such as certain organic and inorganic materials or radioactivity, is also present.

In the case of radiation, this premise appears to have been borne out by our work with inhaled plutonium particles. Following the peripheral pulmonary fibrosis induced by these radioactive particulates, bronchiolar and alveolar proliferation into the scarred areas

occurred. These proliferative and metaplastic cells were subjected to anoxia, necrotic debris, physical irritation, and the carcinogenic influence of the plutonium particles isolated by the scarring process. In addition, there appeared to be a definite migration of particle-bearing macrophages to these scarred areas with time, thus increasing the concentration of carcinogenic material in these areas. The resulting carcinomas were all related histologically to the preceding non-neoplastic lesions, thus emphasizing the importance of fibrosis in the induction of pulmonary tumors under the conditions of this study.

This does not imply that radioatrophy of the lung is the primary cause of the sequential production of radioactive particle-induced lung neoplasms, or that these tumors are the only area of concern following inhalation of these materials. Granted that fibrosis per se is not a significant cause of lung cancer, the fact should not be overlooked that it can be an important contributing factor when a known carcinogen is also present.

SUMMARY

Inhalation of $^{239}\text{PuO}_2$ particles by beagle dogs caused spontaneous death in 37 animals having lung burdens of from 0.5 to 48 μCi . Deaths occurred 2 to 56 months post-exposure and were preceded by progressive lymphopenia and respiratory distress. The pathologic effects observed in the lungs of these animals consisted primarily of severe fibrosis followed by alveolar cell hyperplasia, and bronchiolar and squamous types of metaplasia. Five of the animals showed bronchiolo-alveolar carcinomas, an incidence of 14 percent as

compared to a primary lung tumor incidence of 0.2 percent in canine necropsy material. All neoplasms originated in peripheral lung areas in association with particle retention, fibrosis, and bronchiolo-alveolar metaplasia. The irradiated, proliferating cells, comprising the hyperplastic and metaplastic response to lung scarring, appeared to be the nidus of tumor formation. Metastases to the middle and left bronchial lymph nodes were seen in one dog.

Although fibrosis per se is not a significant cause of lung cancer, it cannot be ignored as an important contributing factor when a known carcinogen is also present.

Fig. 1. Diffuse distribution of plutonium particles in lung immediately following exposure. Autoradiograph, H&E, X 82.

Fig. 2. Bronchiolar epithelial hypertrophy, and edema and inflammatory cell infiltration at 50 days post-inhalation. Autoradiograph, H&E, X 208.

Fig. 3. Squamatization of epithelial lining of terminal bronchiole, 65 days post-inhalation. Autoradiograph, H&E, X 208.

Fig. 4. Mixture of squamous and bronchiolar-type alveolar metaplasia, 65 days post-inhalation. Autoradiograph, H&E, X 208.

Fig. 5. Bronchiolo-alveolar carcinoma with papillary character.
H&E, X 82.

Fig. 6. Bronchiolo-alveolar carcinoma with glandular character.
H&E, X 82.

Fig. 7. Fusiform or spindle-like cells. H&E, X 208.

Fig. 8. Metastases of primary lung tumor to left bronchial lymph node. H&E, X 82.

Fig. 9. Squamous metaplasia of bronchioles. H&E, X 208.

Fig. 10. Heavy concentration plutonium particles in bronchial lymph node 4 years post-inhalation. Autoradiograph, H&E, X 82.

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