Beryllium Carcinogenesis

I. Inhalation Exposure of Rats to Beryllium Sulfate Aerosol

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SUMMARY

One hundred fifty rats (with equal number of controls) were exposed to the inhalation of beryllium sulfate aerosol at a mean atmospheric concentration of 34.25 µg of Be/cu m, and sacrificed in monthly groups during 72 weeks of exposure. Average lung weight towards the end of exposure was 4.25 times normal. Histopathologic examination disclosed 2 gradually developing pathologic processes: (a) an inflammatory response chiefly characterized by marked accumulation of histiocytic elements forming clusters of macrophages in the alveolar spaces; and (b) a proliferative response, progressing from early epithelial hyperplasia of the alveolar surfaces, through metaplasia and anaplasia, to lung cancer. The first tumors were found after 9 months of exposure, the incidence rapidly rising, and reaching 100% at 13 months (vs. 0% in controls). All tumors appeared to be alveolar adenocarcinomas, in some instances with focal intermixure of other types. Out of 56 tumors studied, 3 reached a very large size comparatively early. Females appeared to be more vulnerable to the exposure than males in terms of attritional mortality and body weight loss.

INTRODUCTION

The carcinogenic capacity of beryllium compounds has been of interest to experimental cancer research since Gardner and Heslington in 1946 (7) first produced osteosarcomata in rabbits that had received i. v. injections of zinc beryllium silicate. Similar results were obtained subsequently, with a greater variety of beryllium compounds including also the oxide and phosphate, by Barnes et al. (2), Cloudman et al. (3), Dutra and Largent (5), Hoagland et al. (10), Nash (14), and Vorwald (19). Osteosarcoma of the rabbit was also produced by inhalation exposure to BeO dust by Dutra et al. in 1951 (6), while pulmonary carcinoma of the rat was obtained by intratracheal injection and/or inhalation of BeO and BeSO₄, respectively, by Vorwald in 1953 (20). Further work on the bone tumors of rabbits was reported by Araki et al. (1), Higgins et al. (9), Janes et al. (11, 12), Kelly et al. (13), and Yamaguchi (25); and on the pulmonary tumors of rats by Schepers et al. (17), Vorwald and Reeves (22, 23), and Schepers (15). Recently, Vorvald (21) as well as Schepers (16) also observed pulmonary carcinoma in monkeys exposed to the inhalation of beryllium sulfate and phosphate, respectively. These experiments were reviewed recently by Vorwald et al. (24).

In this series of articles, we shall report the results of an inhalation experiment on 300 rats exposed to aerosolized beryllium sulfate for periods ranging up to 72 weeks. This study was focused on the chain of events leading to the malignant transformation in the lungs, and on the characterization of the obtained pulmonary tumors with respect to pathologic type, biochemical composition, and metabolic behavior. The first of these articles discusses exposure, animal colony management, and histopathologic data.

MATERIALS AND METHODS

Animals. One hundred fifty male and an equal number of female rats, Sprague-Dawley C.D. strain (obtained from the Charles River Breeding Laboratories, Brookline, Mass.) were obtained at the age of 3 weeks and quarantined with prophylactic administration of tetracycline-HCl (2% solution of Polyotic, supplied by the American Cyanamid Co., Princeton, N. J., in the drinking water) for 2 weeks. They entered the exposure chambers at the age of 6 weeks. Additional courses of medication (identical with that in the quarantine period) were administered to the surviving complement of animals during the 67th-68th and 75th-76th week of life, respectively, in order to combat intercurrent respiratory infection. Watering and feeding (Rockland complete rat-mouse diet from Teklad, Inc., Monmouth, Ill.) were ad libitum throughout the experiment.

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Exposure. Two chambers of 2 cu m volume, each holding 6 wedge-shaped cages of about 0.25 sq m floor area, were used. Design of the chambers was based on a successful original model (18), with improvements relative to airflow characteristics and sanitation facilities. Males and females were equally divided between the chambers, placing 25 animals of one sex into each cage, and placing male- and female-containing cages into alternating positions in each chamber. In one of the chambers, a 30% aqueous solution of commercial BeSO₄·4H₂O (from the Brush Beryllium Co., Cleveland, Ohio) was disseminated from a glass aerosol generator, with an airflow of 0.27 cu m/min. The minute droplets of the aerosol evaporated in the chamber atmosphere, so that the material actually inhaled by the animals consisted of microcrystalline particles of BeSO₄·4H₂O.

Thirty five μg Be/cu m air was the target concentration; this concentration was approximated satisfactorily as evidenced by the analysis of 50 air samples, yielding an average value (±1 S.D.) of 34.25 ± 23.66 μg Be/cu m. Electron microscopic study of particle size, based on a sample of about 800 particles, gave an average diameter of 0.118 μ.

In the other chamber, distilled water was disseminated at identical rate in order to duplicate the temperature, humidity, and noise conditions to which the exposed animals were subjected. Thus, the presence or absence of BeSO₄ in the air was the only parameter by which the exposed and unexposed animal groups differed from each other. Dissemination was provided 7 hr daily, 5 days weekly; the complete course of exposure, totaling 2400 hours, was reached in 72 weeks. Temperature in the chambers varied from 24°C to 27°C; relative humidity averaged 75%.

Sacrifices. One animal was taken at random from each cage, totaling 3 male and 3 female rats from both the exposed and control colonies, at each of the monthly sacrifice periods. Scheduled sacrifices were suspended after 56 weeks of exposure, in order to allow a significant number of animals for study at the end of the experiment. At the termination of exposure 16 weeks later, all test animals appeared to be very sick, so that the study was rapidly concluded in 5 weekly sacrifices of 12 animals each. The animals were killed while under Surital (from Parke, Davis & Co, Detroit, Mich.; 3 ml/kg of a 2.5% aqueous solution) anesthesia, the chest cavity opened, blood withdrawn from the heart into a heparinized syringe, and the lungs perfused through the right atrium with 0.9% saline in order to achieve exsanguination. The lungs were subsequently excised, trimmed, blotted between sheets of filter paper until no more moisture was extractable, and weighed to the nearest 0.1 gm (wet weight). The lobes of the lung were examined for gross pathology; neoplasms, pus sacs, and other diseased tissue, if any, were separately dissected and weighed. Specimens obtained for histopathology were fixed in formalin and stained with hematoxylin-eosin.

RESULTS

The sanitary status of the animals during the experiment was excellent, and no animal was lost through attrition during the first year. Mortality due to respiratory or other infection did not appear until 55 weeks of age, and was successfully controlled by antibiotic medication, so that 87% of all animals survived to their scheduled sacrifices. Total attritional death was the following: control males, 8; exposed males, 9; control females, 4; exposed females, 17. These animals were discarded because of potential postmortem changes, and the tissue data reported here are all based on rats sacrificed according to schedule.

Average body weights showed steady increase during the first year of life and reached plateaus at the following values: control males, 625 gm; exposed males, 600 gm; control females, 430 gm; exposed females, 315 gm. It thus appears that females showed...
higher vulnerability to the exposure than males both in terms of attritional mortality and body weight loss.

Wet weight of whole lungs (Chart 1) showed progressively increasing differences between control and exposed animals. Towards the end of the experiment, exposed lungs weighed, on the average, 4.25 times as much as their control counterparts. This marked increase was invariably accompanied by grossly visible changes of tissue texture. Histopathologic examination suggested the existence of two distinct pathologic processes: one inflammatory and one proliferative.

The inflammatory response was chiefly characterized by marked accumulation of histiocytic elements which thickened and distorted the alveolar septa, and formed clusters of macrophages in the alveolar spaces (Fig. 1). Lymphocytic infiltration of the alveolar septa was also prominent, but the latter lesion occurred in control lungs as well: a plot of average severity (visually graded on a scale 0 to +++) showed no difference between the 2 colonies with respect to lymphocytic infiltration, while the accumulation of alveolar macrophages during the experiment was gradual, exposure-dependent, and virtually confined to the beryllium-exposed colony (Table 1). Occasional occurrence of granulomatosis and fibrosis was also seen, but on the whole the incidence of these lesions was insignificant. Emphysema and arteriolar wall thickening occurred more frequently, but without a consistent morbidity trend.

The proliferative response commenced very rapidly after initiation of exposure and consisted, in its first stage, of epithelial hyperplasia involving the alveolar surfaces. Such distinctly visible epithelial investment of alveoli occurred in control animals only during their senescence, and it never progressed to a more advanced proliferative form. In the exposed animals, however, the proliferating epithelium reached the stage of metaplasia during the 26th–28th week of life, exhibiting greater abundance of epithelial cells, frequently forming multilayers (Fig. 2). Ultimately, the process reached the stage of anaplasia during the 38th–46th week of life, with rampant proliferation and incipient loss of pulmonary architecture in the afflicted regions (Fig. 3). The gradual development of these steps, with eventual decrease in the occurrence of the individual stages due to transition of the same cell populations into the next and more advanced proliferative forms, is shown in Table 2.

First appearance of frank and fully developed tumors was observed after 9 months of exposure, with the incidence rapidly rising, and reaching 100% at 13 months. No animal without pulmonary tumor was found among the 43 exposed rats sacrificed on or after that date, while tumor incidence in controls remained 0 throughout the experiment.

Altogether 56 tumors were studied. They all appeared to be adenocarcinomas showing a predominantly alveolar pattern, and multicentricity in origin (Fig. 4). About 1/4 of the tumors also showed relatively small foci where the histologic pattern differed:

1. Focal columnar cell pattern. These cells resembled bronchiolar epithelium; large columnar ciliated cells with malignant nuclei were seen in some areas (2 specimens, Fig. 5).
2. Focal squamous cell pattern. These varied from well-differentiated foci with keratin production to more undifferentiated types (7 specimens, Fig. 6).
3. Focal vacuolar cell pattern. The cytoplasm of the malignant cells had a vacuolated appearance (6 specimens, Fig. 7).
4. Focal mucin-producing cell pattern. Small numbers of malignant cells surrounded by pools of mucin (2 specimens, not shown).

Chart 2 shows tumor size, expressed as % of lung weight. The observed points appear to fit on two distinct curves, and it has been tempting to regard these as "fast-growing" and "slow-growing" branches of tumor development, even though the actual
rate of growth of any single tumor was not ascertainable in this experiment. It is interesting, but in view of the scarcity of specimens not conclusive, that the “fast-growing” branch consists exclusively of females.

**DISCUSSION**

The problems of aerosol generation, atmospheric concentration control, and the treatment of chamber exhaust in order to avoid contamination of the environment and hazard to operating personnel tend to render this type of study cumbersome, and liberal use was made in the conduct of this experiment of the extensive experience of this Department relative to inhalation exposure of rats to aerosolized beryllium salts leading to pulmonary carcinogenesis (A. J. Vorwald and E. C. J. Urban, unpublished data). Of particular importance was the sanitary management of the animal colony, in order to assure survival for a sufficient period and thus to allow the neoplasms to develop.

Chronic pneumonitis with purulent bronchiolitis of apparently bacterial etiology is endemic to the albino laboratory rat, often progressing to form discrete abscesses in various segments of the lung. The observed lymphocytic infiltration of the alveolar septa and possibly the hyperplasia of the alveolar epithelium in exposed as well as control animals are the histopathologic manifestations of this general process. Naturally, the environmental conditions of the chamber and especially the primary irritation exerted by the acidic aerosol (pH of the disseminated BeSO\textsubscript{4} solution was 2.75) tend to precipitate and/or aggravate this condition, and keeping the exposed rats alive may become a major problem. Selection of a hardy and yet susceptible animal strain, judicious control of aerosol concentration designed to avoid insufficient stimulus as well as acutely toxic overexposure, meticulous sanitation practices including efficient waste disposal and periodic sterilization of cages, and the prophylactic administration of antibiotics are equally important measures of epizootic control, and essential prerequisites of an adequate carcinogenic challenge.

Even though the initial proliferative response to beryllium exposure occurred early, the development of tumors required considerable time, and the interval of 9–13 months is in agreement with the results of earlier work (20, 22). Occurrence of the noted focal histologic variants notwithstanding, in our opinion the basic pathology of these cancers is probably uniform, and fundamentally of the adenomatous type. Classification into numerous distinct varieties has been attempted (17), but these may reflect more the incidental local modifications in the overall histologic picture rather than being a manifestation of altogether different pathologic processes. Most of the early tumor foci seen in this study appeared to be alveolar rather than bronchiolar. This finding is consistent with the expected pathogenesis of these neoplasms; the submicron particle size of the aerosol certainly assured essentially complete penetration of the inhaled material throughout the respiratory system. Permanent deposition of beryllium was more likely on the alveolar epithelium than on the bronchiolar epithelium, since the former is not ciliated. Consequently, the alveolar cells may be presumed to have had greater exposure to the challenge and were thus more likely to initiate the malignant transformation. The tumors showed a high degree of local invasiveness; metastatic spread to the tracheobronchial lymph nodes and the pleura (20), as well as successfully accomplished serial homotransplants (17) were reported earlier.

The apparent difference in the susceptibility of the sexes, manifested as higher attritional mortality and body weight loss as well as exclusive incidence of the early large tumors in females was not reported earlier, and the latter phenomenon did not occur in enough animals to establish its numerical significance. However, the excess attritional losses were about equally divided among the three alternately placed cages housing the exposed females, and thus they are not explainable on the basis of random infection spreading in one colony. It is interesting to remember that the attack rate of industrial berylliosis in humans is also reported to be higher in women than in men (4, 8).

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Fig. 1. Specimen No. 815-19. Lung of male rat, age 42 weeks, exposed to inhalation of BeSO₄ for last 8 months. Inflammatory response is shown by the marked accumulation of histiocytic elements, forming clusters of macrophages in the alveoli. × 250.

Fig. 2. Specimen No. 815-71. Lung of male rat, age 46 weeks, exposed to the inhalation of BeSO₄ for last 9 months. Proliferative response is shown by the atypical hyperplasia and metaplasia of the epithelium which lines a respiratory bronchiole, an alveolar duct, and the contiguous alveoli. × 100.

Fig. 3. Specimen No. 815-79. Lung of male rat, age 62 weeks, exposed to the inhalation of BeSO₄ for last 13 months. Advanced anaplastic changes of the respiratory epithelium are shown, bordering on frank malignancy. × 100.

Fig. 4. Specimen No. 815-81. Lung of male rat, age 78 weeks, exposed to the inhalation of BeSO₄ for last 16 months. The predominant pattern of adenocarcinoma is shown, with variable epithelial features. × 100.

Fig. 5. Specimen No. 815-81. Detail from the same lungs as that shown in Fig. 4. Focal area of columnar cell pattern within the adenocarcinoma. × 250.

Fig. 6. Specimen No. 815-41. Lung of male rat, age 82 weeks, exposed to the inhalation of BeSO₄ for 16 months, and subsequently living in clean air for 1 month. Focal area of squamous cell pattern within the adenocarcinoma. × 100.

Fig. 7. Specimen No. 815-120. Lung of female rat, age 54 weeks, exposed to the inhalation of BeSO₄ for last 11 months. Focal area of vacuolar cell pattern within the adenocarcinoma. × 100.

Fig. 8. Specimen No. 815-131. Lung of male rat, age 78 weeks, exposed to the inhalation of BeSO₄ for last 16 months. Focal area of undifferentiated cell pattern within the adenocarcinoma. × 100.

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