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THE BIOLOGICAL EFFECTS OF BERYLLIUM

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The unusual variety of effects which beryllium exhibits in biological systems has not been generally appreciated. Beryllium has the capacity to produce in humans: granuloma of the lung and skin, acute pneumonitis and allergic dermatitis; and in animals: sarcoma of bone, carcinoma of the lung, rickets, osteosclerosis, inhibition of limb regeneration in larval Amphibia, and macrocytic anemia. Beryllium also inhibits a number of enzyme systems including alkaline phosphatase. The diversity of these effects suggests that further research on beryllium may uncover metabolic disturbances common to some of these very dissimilar responses.

The purpose of this paper in reviewing the current status of beryllium research is to interest investigators, particularly those working on chronic inflammatory reactions, cancer, bone metabolism, immune reactions and enzyme chemistry, in some of the interesting facets of the beryllium field which bear on their work.

Beryllium Diseases

The beryllium diseases in humans include: acute conjunctival, nasopharyngeal and tracheal irritation, acute pneumonitis, pulmonary granulomatosis (chronic berylliosis), subcutaneous granuloma, cutaneous ulcer, and contact dermatitis. A number of good reviews of the clinical characteristics of these diseases have been published (1, 2, 3). Since this paper is primarily concerned with the research aspects of the problem, only the pertinent facts of the clinical experience will be discussed.

Chronic Berylliosis

Beryllium has produced about 300 cases of chronic pulmonary granulomatosis (4). The disease was initially confused with Boeck's sarcoid until epidemiological evidence made this diagnosis untenable. The symptoms of chronic berylliosis follow a latent period of 1 to 11 years and in a number

Epidemiologically, chronic berylliosis has some unusual features (12). Persons inhabiting the immediate neighborhood of a beryllium refinery were exposed to extraordinarily low atmospheric concentrations of beryllium, (approximately 1 ug/cubic meter) and sustained an incidence of 1%. This was somewhat higher than the incidence within the plant (1/2 per cent) where exposure levels were a thousand-fold higher. Moreover, those workers who contracted the disease within the plant were employed for six months or less. Most cases of chronic berylliosis occurred in industries handling beryllium phosphors. In one fluorescent lamp factory, the incidence is now 7-10% (13).

This plant had utilized a fluorescent phosphor ($ZnBeMg(SiO_4)Mn$) containing 12% beryllium oxide. The oxide concentration was reduced to 2% in 1942 and few if any cases occurred in persons exposed solely to the lower concentration. Beryllium oxide has been suggested as the etiological agent because it is the one compound to which most cases of berylliosis have been exposed. However, the incidence among workers handling the phosphors has been so much higher than other groups that one or more components of the phosphors may be of etiological importance.

Acute Pneumonitis

A single large exposure to beryllium fluoride has been observed to produce symptoms of pneumonia in seventy-two hours (14). However, lower exposures to soluble beryllium salts and finely divided beryllium oxide have produced gradually increasing respiratory symptoms over the course of weeks. A sharp decrease in vital capacity usually precedes the characteristic dyspnea and cough (15). The illness lasts from one to four months and in one series of ninety-three cases, 10% died and none progressed to the chronic disease. However, a few cases have apparently developed chronic berylliosis after acute pneumonitis (4), but the two diseases are uncommonly associated.

The typical pathology of acute pneumonitis is characterized by an acute inflammatory reaction of the bronchioles with alveolar exudation containing numerous monocytic and phagocytic cells (11). There have been all gradations in the pathology between the acute and chronic forms of berylliosis including small granulomatous nodules which appear in the late stages of the acute disease. A few cases of acute pneumonitis have been treated with ACTH with apparent benefit but there has not been sufficient experience to evaluate this therapy (3).

Beryllium Skin Lesions

Beryllium phosphor embedded under the skin in some humans produces, after a latent period of several months, a granulomatous nodule resembling the pulmonary lesion of berylliosis and sarcoid (16). There have been no reports of the effect of ACTH on the lesion and it is cured by excision. Soluble beryllium salts embedded in skin abrasions produces a superficial chronic ulcer which also does not heal unless excised (2).

Contact dermatitis is common in the extraction industry and usually follows initial skin contact after a period of 3-10 days (17). Patch tests with soluble beryllium salts have been positive, indicating that the dermatitis has an allergic basis. Beryllium fluoride has been found to be the most potent sensitizing agent in patch tests and likewise, its dermatitis is the most difficult to treat.

Beryllium Research

There are a number of facets to the beryllium research program. From the practical standpoint, an understanding of the genesis of the various beryllium diseases is prerequisite to an adequate preventive and therapeutic program. On the other hand, the close resemblance between chronic

berylliosis and Boeck's sarcoid extends the significance of beryllium research into the field of granulomatous diseases where information is badly needed. The ramifications go further since beryllium in animals produces cancer of bone and lung, rickets, osteosclerosis, anemia, interference with regenerative processes, as well as inhibiting a number of enzyme systems. The metabolic interrelationships of these reactions may prove to be of considerable theoretical importance.

The pathogenesis of chronic berylliosis has been given considerable thought. Since berylliosis does not follow the usual pattern of chemical toxicity, it has been postulated that the disease represents an immunological reaction (12) on the following basis: the incidence under most circumstances is very low (only a few per cent), suggesting that those people who develop the disease have a constitutional susceptibility. In one beryllium refinery and its environs, there was an inverse relationship between dosage and disease incidence. The latent period is unusually long, one to eleven years. Beryllium does produce an allergic reaction in the skin and therefore it may have the same potentiality for the lung. Finally, granulomatous reactions are sometimes seen under conditions suggestive of an immunological reaction, as in tuberculosis.

The findings of minute amounts of beryllium in the lungs of some fatal cases of berylliosis (1 microgram/100 grams) (11) suggests that an alteration in the usual enzymatic behavior of the lung tissue could be responsible for the disease process. There is suggestive evidence for this possibility in the number of enzymes which beryllium does inhibit in vitro.

Chronic berylliosis has not yet been reproduced in animals. A variety of species, including rats, mice, rabbits, guinea pigs, hamsters,

and a few cats, monkeys and pigs have been exposed to a number of beryllium compounds: sulfate, metal, hydroxide, oxide, stearate, carbonate, fluoride and oxyfluoride, and beryllium phosphor (18). Severe chronic inflammatory reactions have been produced in the rabbit and guinea pig with intratracheal injections of the oxide, hydroxide, stearate and the phosphor, but the lesions were not considered typical beryllium granulomas. It has been reported that granulomas are produced in implants of embryonic mouse lung tissue by beryllium oxide (19). However, these lesions resemble the reactions seen with intratracheal injections in adult animals (9).

One strain of rats, exposed for twelve to eighteen months to beryllium oxide and beryllium sulfate dust, exhibited an increase in the spontaneous incidence of small discreet granulomas from 2-3% to 100% (9). In addition, the number of lesions per frontal section of both lungs is increased from 2 to 3 to about 15. As might be expected from the small number of lesions, none of the animals exhibited symptoms of pulmonary insufficiency. Therapy with ACTH for a period of several months is said to cause a temporary regression in these lesions.

To obtain a chronic beryllium lesion in animals is, of course, an important initial step in studying the pathogenesis of pulmonary granulomatosis. As is apparent from the above discussion, the problem has not been satisfactorily solved. The first attempts at reproducing chronic berylliosis were unsuccessful but some of the rabbits developed osteogenic sarcoma (20, 21). This has been repeatedly confirmed in the rabbit by intravenous or intraperitoneal injection of soluble beryllium salts, the beryllium phosphor and the beryllium metal. It also occurred following the inhalation of beryllium oxide (22), where the bone concentration of

beryllium averaged 35 micrograms per 100 grams of bone. Thus far, bone sarcoma has been observed only in the rabbit. Recently, carcinoma of the lungs has been produced in rats exposed to beryllium sulfate and beryllium oxide dusts for a period of nine to twelve months (23).

The only human granulomatous lesion which has been convincingly reproduced in animals is the skin granuloma. Subcutaneous implantation of beryllium phosphor in the porcine pig stimulates the formation of a granuloma which resembles the human lesion closely although somewhat more fibrotic (24). Implantation of beryllium oxide alone produced only a non-specific granuloma which regressed spontaneously. The production of this lesion has been recently confirmed (25).

It has been reported that, of sixty elements, beryllium was the only one to have a significant effect on tuberculosis in guinea pigs (26). The number of tuberculous lesions seen grossly in the lung was markedly increased by injections of beryllium salts. While the response is unconfirmed, the relationship is interesting because of the unusually high incidence of tuberculosis in cases of Boeck's sarcoid and the similarity of chronic berylliosis and sarcoid. There have been no reported studies of the tuberculin reaction in chronic berylliosis although a few cases are said to have had negative responses (4).

The complexity of beryllium chemistry in aqueous solution has been a barrier to the study of the hyperimmune state in berylliosis as well as enzymatic inhibition by beryllium. In the physiological range of pH, beryllium probably exists in the blood as a citrate complex (27) and to a lesser extent as malate or bicarbonate. The amount of ionized beryllium in blood is probably less than 10^{-10} molar.

Significant complexing of beryllium with protein has not been observed except in the presence of fluoride (28). Beryllium also reacts strongly with ribosenucleic acid, presumably with its phosphate radicals, but not with desoxyribosenucleic acid (27). The potentiation by hydrogen fluoride of the acute pulmonary toxicity to soluble beryllium salts (29) may be explained by the increased binding of beryllium with protein due to a linking effect of fluoride. Likewise, of the soluble salts, beryllium fluoride is the most potent sensitizing agent in skin.

Beryllium has been demonstrated to inhibit alkaline phosphatase (30, 31, 32, 33), adenosine triphosphatase (32, 34), and phosphoglucomutase (34). The inhibition of alkaline phosphatase has been repeatedly confirmed, but most of the enzyme studies suffer from the lack of detailed information on the concentration of ionized beryllium involved in the reactions.

Beryllium has been observed to substitute to a limited extent for magnesium in plant growth and to inhibit plant alkaline phosphatase (35). Magnesium-beryllium antagonism could not be demonstrated in vitro although it has been postulated that the inhibition of alkaline phosphatase by beryllium is due to competition with magnesium. However, a certain degree of neutralization of beryllium-induced mitotic abnormalities was affected by magnesium (36).

Osteosclerosis has been observed consistently in rats and rabbits following parenteral injection of beryllium salts (37). This lesion is characterized by an excessive proliferation of spongy bone within the marrow cavity. A few cases of chronic berylliosis have exhibited dense bones (4) but no systematic radiological or pathological survey has been made. Beryllium, fed to animals in large quantities, produces a phosphate

deficiency rickets which is largely preventable by simultaneous administration of phosphate compounds (38). The high incidence of renal stones in the chronic berylliosis, has not been reported in animals. Administration of parathyroid hormone does not prevent the osteosclerotic lesion or cause its regression (39), but it does diminish the deposition of beryllium.

Aurintricarboxylic acid has been found to be an effective antidote to the acute poisoning produced by intravenous doses of beryllium sulfate (40). This effect is also observed with the salicylic acid portion of the aurintricarboxylic acid molecule (41). However, thirty-five times more salicylate is required for equivalent protection on a molar basis. The action of both substances with beryllium is presumably due to chelation. Salicylates raise the urinary excretion of beryllium in contrast to aurintricarboxylic acid (42).

Exposure of dogs and rats to beryllium sulfate mist results in a macrocytic hyperchromic type of anemia (43). Folic acid had no effect on the anemia of dogs but did prevent its occurrence in rats. Studies utilizing alpha C^{14} acetate indicate that hemin and globin synthesis are depressed but the latter is more markedly affected (44). The nature of the metabolic disturbance in this response is not understood.

Discussion

One of the most difficult aspects of the beryllium program has been the inability to reproduce in animals the clinical syndrome of chronic berylliosis. Perhaps in the species already studied, the susceptibility to the disease is very low and consequently only guinea pigs, rabbits, rats and mice have had an adequate trial. The capacity of the porcine pig to respond to implanted beryllium compounds with a skin granuloma suggests that it might be susceptible to the pulmonary disease. Because of the

difficulties in handling this animal in exposure experiments, few pigs have been used and with negative results. However, there is now a strain of miniature pigs developed at the Hormel Institute which is more suitable for experimental work. If the allergic hypothesis for chronic berylliosis has merit, the method of exposure of animals on a five-day per week basis for six hours per day may not be the most suitable technique for producing the disease. It is known that under certain circumstances, large doses of antigen suppress the allergic response (45). It therefore might be more effective to expose animals to small amounts of beryllium compounds on an intermittent basis.

The close resemblance between chronic berylliosis and Boeck's sarcoid is one of the most intriguing aspects to the whole beryllium field and suggests that the pathogenesis of both diseases involves similar mechanisms. The etiology of sarcoid is unknown but its relationship to tuberculosis, if elusive, is highly suggestive. The capacity of beryllium to worsen the course of tuberculosis in guinea pigs, although unconfirmed, suggests a possible common denominator in tuberculosis to sarcoid and berylliosis. Unfortunately, there has been no detailed study of tuberculin reactions in cases of chronic berylliosis. Hypercalcemia has been observed in Boeck's sarcoid inconstantly related to hypercalcuria (46). This has been reversed by ACTH but such a response has not been reported in berylliosis. Gramulomas are not uncommonly seen in the bones of patients with sarcoid, unlike berylliosis, whereas an excessive incidence of renal stones occurs in berylliosis but not in sarcoid. However, sarcoid patients occasionally die of renal failure with marked renal hypercalcification (47). It would seem that parallelism between the two diseases extends to abnormalities

in calcium metabolism.

Basic to the problem of both sarcoid and berylliosis, is the general nature of the granulomatous response. In the older studies on the tubercular granuloma, the theory was advanced that in the presence of indigestible material, macrophages become indolent and form the characteristic cells of the lesion (48). This raises the question of whether beryllium in some way interferes with the metabolism of macrophage cells and produces the same effect. It is also of interest that certain phospholipids are very effective in producing experimental granulomata (49) and that beryllium has a high affinity for phosphate compounds.

Although it is true that beryllium interferes with alkaline phosphatase, this enzyme appears to be absent in non-beryllium granulomata, i. e., sarcoid and Hodgkins Disease (50). Unfortunately, there is little published data on the effect of beryllium on acid phosphatase. Since the conversion of monocytes to macrophages in vitro is associated with the appearance of high intracellular acid phosphatase activity (51), a study of the effect of beryllium on this conversion would be most interesting.

Perhaps osteogenic sarcoma, osteosclerosis and pulmonary granuloma are varients of a similar metabolic disturbance in tissues having a common mesenchymal origin. All three lesions seem to represent a proliferative response. According to Haddow, cancer may be a reaction of escape to a primary inhibition of growth induced by the carcinogen (52). A growth depressing effect of beryllium is observed in the inhibition of tissue regeneration in larval Amphibia (53, 54) and the chronic industrial skin ulcer. It would be interesting if an initial growth depression of bone fibroblasts in the presence of beryllium is followed by sarcomatous

degeneration as observed with methylcholanthrene (55).

The presence of a hyperimmune state to beryllium, although postulated, has never been defined. Most patients with chronic berylliosis have positive patch tests to beryllium (3). However, skin sensitivity has been seen in acute berylliosis and these individuals rarely, if ever, develop the chronic disease. Moreover, the positive patch test is not convincing evidence of a hyperimmune state in the lung. Acute berylliosis can be readily produced in animals and might be useful for immunological studies.

The minute quantities of beryllium found in the lungs of individuals with chronic berylliosis raises the question of the relationship of beryllium concentration to the initiation and maintenance of the granulomatous lesion. Evanescent skin granulomata occur in cases of berylliosis but the beryllium content of these lesions is not known. The two phases of the reaction may be relatively independent as seems to be the case with the beryllium bone sarcoma since beryllium is not detectable in the sarcomatous tissue (21). If the subcutaneous granuloma in the pig could be produced by repeated injections of soluble salts of radioactive beryllium, the relationship between the histological appearance of the lesion and the concentration of beryllium could readily be followed. In addition, it would be of interest to determine whether soluble magnesium salts would prevent the appearance of the granuloma in view of the postulated beryllium-magnesium antagonism.

Unfortunately, it is not feasible to use radioactive beryllium for radioautography since the only available isotope, Be^7, emits gamma rays. Localization of beryllium within the cells would be of considerable interest because of the affinity of beryllium for ribosenucleic acid rather than desoxyribosenucleic acid. The problem may possibly be attacked with dyes

such as the naphthachrome series or by use of the fluorescence reaction with morin. There is suggestive evidence that the conchoidal bodies in chronic berylliosis contain beryllium (56). Microdissection and spectrographic analysis of the material in crystal clefts and conchoidal bodies would be difficult but useful.

Parathyroid hormone administered in large doses does not prevent osteosclerosis (39). This suggests that beryllium may block parathyroid hormone perhaps by its inhibition of alkaline phosphatase, thereby producing the excessive bone formation. Presumably, osteosclerosis is caused by excessive formation of osteoid material onto which calcium salts are deposited. If this were so, beryllium should be demonstrable within the osteoid tissue. It might be possible to localize beryllium within the osteosclerotic lesion by decalcifying a section of bone with versene and then analyzing the residual osteoid tissue. It is difficult to see how the beryllium could produce osteosclerosis if its presence in bone were accounted for solely by adsorption onto apatite crystals.

The adrenal hormones are the only effective therapeutic measures used in chronic berylliosis. However, other techniques might be considered. Some individuals with chronic berylliosis have a constant, though small, urinary excretion (57), indicating that beryllium is transported in the blood. This may possibly be the method of distributing beryllium to all portions of the lung and thereby maintaining the concentration high enough to perpetuate the granulomatous lesion. The occurrence of skin granulomas in cases of chronic berylliosis might be an example of this phenomenon. Salicylates form a soluble chelate which is readily excreted in the urine (41). A constant level of salicylates in the blood might serve to interrupt

re-exposure of the lung and may accelerate the slow drainage of the body stores of beryllium. Removal of beryllium from the bone in humans may be of considerable importance if the incidence of bone tumors in beryllium workers becomes abnormally high. Animal studies indicate that up to 60% of the body burden of beryllium can be carried by the skeleton (58). Mobilization of calcium by versene might release that portion of the skeletal beryllium adsorbed onto apatite crystals to be chelated and excreted by salicylates. Parathyroid hormone may help to remove beryllium from the bones in humans as it does to a certain extent in the rat. As another approach, particles of beryllium once deposited in the lung may be rendered inactive by combination with aurintricarboxylic acid inhaled as a mist, thus protecting the lung from further damage.

Summary

The appearance of chronic berylliosis in industrial workers has stimulated a considerable amount of research on the biological effects of beryllium resulting in a number of very interesting and diverse observations. The capacity of beryllium for producing granulomatous disease, cancer, skin allergy and disturbances in bone metabolism suggests that beryllium will be a useful tool for exploring the metabolic interrelationships of these lesions.

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