Beryllium and Growth

I. Beryllium-induced Osteogenic Sarcomata*

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The pathological changes in animals resulting from the administration of compounds of the element beryllium are receiving increased attention because of the widespread use of the metal in industry and the resultant pulmonary and dermatological diseases in industrial workers.

Great impetus was given to the biochemical study of this metal by the important discovery in 1946 by Dr. Leroy Gardner that the intravenous administration of certain beryllium-containing powders to the rabbit led to the development of osteogenic sarcomas (3). The untimely death of Dr. Gardner prevented publication of the extensive data from this work. Since the time of his original observations, the production of these tumors has been confirmed in this laboratory, and it is felt that a brief account of the findings is warranted to emphasize the importance of beryllium in the field of cancer research.

METHODS

The following compounds were used for injection after being finely ground in a ball mill for 8 hours. These powders are virtually insoluble in water.

1. Beryllium phosphate. A 0.1 per cent suspension in saline was administered to five rabbits.

2. “Zinc-beryllium-silicate,” a fluorescent powder containing the oxides of zinc (60 per cent), silicon (30 per cent), manganese (2 per cent), and beryllium (0.3 per cent), as well as traces of other metal oxides (SE-10). Six rabbits received this powder as a 1 per cent suspension in saline.1

3. A similar material containing 14 per cent beryllium oxide and 48 per cent zinc oxide (SE-6-21). This was given to four animals as a 1 per cent suspension in saline.1

4. Beryllium oxide. Four animals received C.P. beryllium oxide (Eimer and Amend), and five received a beryllium oxide preparation used by Dr. Gardner in his experiments, both as 1 per cent suspensions in saline.1

Twenty-four rabbits of unselected strains, 13 females and 11 males, with an average initial weight of 5½ pounds, were used as experimental animals. They were kept in wire cages and fed Purina Rabbit Meal and lettuce.

The powders were injected under sterile precautions into ear veins, in doses of 5 cc. at 1-day or at 4-day intervals, in an attempt to administer a total of 1 gm. of powder.

RESULTS

Seven of the 24 rabbits developed osteogenic sarcomas, and one (which received 100 mg. of beryllium phosphate) is living and well 2½ years after injection. Of the sixteen remaining rabbits, eight died within 3 months of the start of injections, of a multiplicity of causes not related directly to beryllium; and eight died from 14 to 28 months after injections. Most of this latter group had extensive fungus disease of the ears, complicated by pneumonia—the usual cause of death; and seven that had received beryllium oxide had fibrotic changes in the liver, a lesion which is described below. The relation of this lesion to mortality cannot be evaluated from the data.

The pertinent data on the seven animals which developed tumors are presented in Table 1.

Description of disease and pathological findings.—There was no apparent correlation between the type and the amount of material injected, the sex of the animal, and the degree of malignancy of the tumor. The data suggest that, in this study, beryllium oxide was less effective in producing tumors than the zinc-beryllium silicate. The failure of

1 We wish to thank the Saranac Laboratories for generously supplying these materials.

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beryllium phosphate to induce tumors was probably due to the small quantity used.

The earliest signs of the disease were usually a precipitous drop in body weight, accompanied by marked crippling and by trophic changes in the skin of the affected extremity. Among the animals which died spontaneously or which were killed when obviously near death, the duration of symptoms and positive x-ray findings was from 1 to 2 months. X-rays were taken during the injection period and 6 months later, and after that as indicated by evidence of weight loss or other signs of sarcoma. The x-rays clearly demonstrated the inception and progression of the lesions (Figs. 1, 2, and 3).

This phenomenon may be seen in the upper end of the humerus of rabbit No. 7, on the side opposite the tumor (Fig. 2). This change was not noted in the group which did not develop sarcomas, nor was there any evidence that it represented a premalignant change in the sarcoma group.

At death, the rabbits were always extremely emaciated (sometimes weighing only about one-half their maximum weight). The primary tumors were obvious on superficial examination and had usually completely compromised the function of the extremity. The cut surface of the tumors was osseous in some cases (No. 98), whereas in others it showed no gross evidence of calcification (No. 21). There were all gradations between these extremes. Areas of necrosis and hemorrhage were frequent. Note the extensive involvement shown in Figure 4.

Microscopically, these tumors were highly invasive in appearance, and the shaft of the bone at the site of origin was sometimes completely destroyed. Very little cellular or fibrous reaction to the tumor was noted. The cellular picture was that of osteogenic sarcoma, varying from extreme anaplasia to well differentiated, bone-forming tumor. Anisonucleosis, nuclear hyperchromatism, increased mitotic activity, accumulations of multinucleated giant cells, and some evidence of bone matrix formation were present in almost all sections. The accompanying figures (5, 6, and 7) are self-descriptive. Metastatic areas were generally less differentiated than the primary tumor.

The livers from all animals which had received beryllium oxide, and to a lesser extent from the silicate-injected animals, showed a fine white motting throughout which coalesced at the liver edges and in the septa into bright white contracted areas.

### TABLE 1

<table>
<thead>
<tr>
<th>Rabbit (No.)</th>
<th>Compound</th>
<th>Amount of compound (mg.)</th>
<th>Injection period (weeks)</th>
<th>Latent period (months)</th>
<th>Site of primary tumor</th>
<th>Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>ZnBeSiO₄ (2.3 percent BeO)</td>
<td>850</td>
<td>8</td>
<td>13</td>
<td>Femur</td>
<td>Lungs, skull, and ribs</td>
</tr>
<tr>
<td>6</td>
<td>ZnBeSiO₄ (4.3 percent BeO)</td>
<td>450</td>
<td>8</td>
<td>24</td>
<td>Femur</td>
<td>None discovered</td>
</tr>
<tr>
<td>7</td>
<td>ZnBeSiO₄ (2.3 percent BeO)</td>
<td>600</td>
<td>9</td>
<td>15</td>
<td>Humerus</td>
<td>Lungs, ribs, liver, kidney, skin, and omentum</td>
</tr>
<tr>
<td>11</td>
<td>ZnBeSiO₄ (14 percent BeO)</td>
<td>200</td>
<td>0.5</td>
<td>11</td>
<td>Tibia</td>
<td>Lungs, liver, kidney, and omentum</td>
</tr>
<tr>
<td>21</td>
<td>ZnBeSiO₄ (14 percent BeO)</td>
<td>250</td>
<td>2</td>
<td>21</td>
<td>Femur</td>
<td>Lungs, liver, kidney, and omentum</td>
</tr>
<tr>
<td>22</td>
<td>ZnBeSiO₄ (14 percent BeO)</td>
<td>230</td>
<td>2</td>
<td>17</td>
<td>Femur</td>
<td>None discovered</td>
</tr>
<tr>
<td>98</td>
<td>BeO</td>
<td>1,000</td>
<td>16</td>
<td>20</td>
<td>Ischium</td>
<td>A few tiny nodules in liver and lungs</td>
</tr>
</tbody>
</table>

![Fig. 1.—Rabbit No. 5. A. X-ray 18 months after injections. An early sarcoma may be seen on the medial condyle of the left femur; B. 1 month later the tumor has grown to immense proportions, and extensive metastases were visible in the lungs at this stage.](image-url)
Fig. 2.—Rabbit No. 7. A. X-ray 15 months after the injection of beryllium—note the extensive destruction of the humerus by the sarcoma and the increased density of the bone of the upper half of the opposite humerus; B. numerous metastases are evident in the lungs, liver, and the peritoneal cavity.

Fig. 3.—Rabbit No. 98. A. Early sarcoma appearing on the ischial tuberosity 20 months after the injections; B. 1 month later the tumor has reached a large size. Very early metastases were found at autopsy.
(Fig. 8). Microscopically, accumulations of portal fibrous tissue, proliferation of bile duct epithelium, and collections of amorphous particulate material (which appeared to be the injected powder) were evident (Fig. 9). Similar accumulations of particles were found in the spleen. These changes were as commonly found in those animals which failed to develop tumors as in those which developed tumors.

One rabbit (No. 95), which received beryllium oxide but did not develop a tumor, had a small spleen, the Malpighian corpuscles of which were filled with large, multinucleated giant cells. These cells were loaded with particles (Fig. 10).

**Transplantation**—Several attempts were made to transplant the sarcoma tissue. Using aseptic precautions, fresh tissue was cut into small pieces in saline and implanted in the following sites: tumor tissue from one rabbit was introduced by trocar directly into the distal femoral marrow cavity of two normal rabbits; tissue from two other tumors was introduced into the anterior chamber of the eyes of four guinea pigs and also under the skin of two rabbits; finally, tissue from another rabbit was implanted into the anterior chamber of a normal rabbit's eye. All these transplanted tissues failed to grow.

**Alkaline phosphatase studies.**—Alkaline phosphatase determinations on tissues and serum were done in a manner similar to that described by Franseen and McLean (2), with minor modifications. Studies on activation and inhibition of the enzyme were performed as described in a previous paper (4). A selection of relevant data on tumor-bearing rabbits is presented in Table 2.

The range of serum alkaline phosphatase activity in six rabbits (four rabbits which never developed tumors and two rabbits in the pre-tumor stage) was 0.4–2.1 units per cubic centimeter of serum. In the two animals in which phosphatase determinations were done when the sarcoma was just visible by x-ray (Nos. 6 and 7), the values were slightly but significantly elevated. Thereafter, there was a rapid and dramatic rise in activity, which seemed to parallel the extent of metastases rather than the size of the primary lesion. There was no rise in serum phosphatase in animals with no tumor.

Tissue alkaline phosphatase determinations revealed a high activity in the tumor tissue in every instance (Table 2). The areas of dense bone described earlier and of bone adjacent to the tumor showed significant elevations in activity.

Because of the known inhibitory effect of beryllium upon the activity of alkaline phosphatase (1, 4, 5), a study of the effect of beryllium upon the phosphatase of this particular tumor was undertaken. The results of this study clearly showed that the glycerophosphatase of dialyzed Be-sarcoma extracts was activated by magnesium and inhibited by beryllium in a manner identical with that described for other tissues (4) over a pH range of from 7 to 10.

**DISCUSSION**

The production of an extremely malignant tumor with a simple metallic oxide is of fundamental importance in the field of cancer research. That beryllium itself is a true sarcomagenic agent is strongly suggested by the fact that, as shown by Gardner (3), beryllium phosphate, as well as the oxide and silicate, is an effective tumor inducer. It is not known whether a local physical irritation factor contributed by an insoluble powder is an adjuvant to the chemical action of ionic beryllium. Soluble salts of beryllium have not produced tumors, but to do this would involve long-term injections of beryllium in small doses, a tedious procedure which has not been undertaken. Beryllium compounds have been injected into rabbits, rats, and guinea pigs, and of these the rabbit is apparently the only susceptible species. Elucidat-

2 D. M. Tibbetts, unpublished material.
Fig. 5.—Rabbit No. 7. A section of a kidney metastasis showing tumor tissue invading the kidney cortex and laying down some bone matrix. × 100.

Fig. 6.—Rabbit No. 21. Metastatic area in the liver illustrating a high degree of anaplasia. Note the giant cell, anisonucleosis, and hyperechromatism. × 600.

Fig. 7.—Rabbit No. 98. A miliary metastatic nodule in the liver. This was one of the few metastases found in this animal and was derived from a primary lesion which was more differentiated than most of the other tumors. × 100.
is inhibited by beryllium in a manner similar to normal bone phosphatase suggests that this enzyme is not altered in its properties. However, our criteria for characterizing phosphatases are so limited that it is impossible to say at this time whether or not the enzyme is different. The finding does not exclude the possibility, however, that the action of beryllium upon normal bone phosphatase may have been initially important in tumor induction. Elucidation of the mechanism involved here must await clarification of the significance of phosphatases in general. Evidence is accumulating that beryllium has no similar inhibitory effect upon a wide variety of other essential magnesium-
activated enzymes, and there is a growing respect for the importance of phosphatases in protein metabolism and the growth processes. One might assume that the phosphatases control the level of organic phosphates which store energy for synthesis and that any agent which selectively inhibits the hydrolytic action of these enzymes might be expected to increase synthetic activity. However, evidence is now appearing (6) that alkaline phosphatase has the ability to transfer phosphate from one organic molecule to another, an effect which might have the opposite significance in synthesis.

It is the purpose of the present investigations in these laboratories to correlate such specific biochemical actions as these with a variety of growth responses to this metal.

SUMMARY

1. Twenty-four rabbits were injected with insoluble beryllium compounds, and seven animals developed osteogenic sarcomas in from 11 to 24 months after injection. These tumors were highly malignant and metastasized rapidly. Seven other injected rabbits—apparently dying of unrelated causes—were found to have remarkably fibrotic and contracted livers.

2. All attempts to transplant these tumors met with failure.

3. The serum alkaline phosphatase activity rose rapidly, paralleling the spread of the tumor throughout the body, and the activity of this enzyme in the tumor tissue itself was extremely high. Magnesium was found to activate, and beryllium to inhibit, the phosphatase associated with the sarcoma in the same manner that these metals affect normal alkaline phosphatase.

4. The importance of an extremely malignant sarcoma produced by a simple metal oxide, in the light of the known effects of beryllium on certain enzyme systems, is discussed.

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