BERYLLIUM-INDUCED OSTEOGENIC SARCOMA IN RABBITS

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During an extensive experimental study of ultrasound* we learned that such energy can inhibit the growth of bone in animals. Obviously the use of ultrasound on bone tumours presented itself as a potential therapeutic procedure. Since a method for producing such tumours with beryllium was available we endeavoured to produce in rabbits a series of such tumours upon which the effects of ultrasonic energy might be studied. As we proceeded with the work of producing and studying such tumours certain pathological changes were observed, particularly in the spleen, which seemed of sufficient interest to justify a separate report.

This report, then, is restricted to a consideration of pathological changes developing in the organs of the rabbit after intravenous injections of beryllium material. Particular emphasis is given to the pathological changes which accompanied the development of bone tumours, and to the apparent relationship between tumour-genesis in the bones and atrophy of the spleen.

BACKGROUND INFORMATION

Beryllium, a rare metallic element, was discovered by Vauquelin about 1798 and was named glucinium because certain of its salts have a sweet taste. In nature it occurs in combination with other elements. It is a hard, grey metal with an atomic number of 4, atomic weight of 9.02 and specific weight of 1.86. It has become important industrially as a constituent of alloys with copper, aluminium, magnesium, nickel, steel and silver. The alloy known as beryllium-copper is resistant to corrosion, non-magnetic, non-sparking, highly resistant to fatigue and with a remarkably high electrical conductivity. Beryllium is used industrially in precision instruments, non-sparking tools, aeroplane fittings, and electrical equipment, as well as in many other applications. The fluorescent property of certain beryllium compounds has application to the fluorescent-lamp industry.

It is most important that all who use beryllium and its compounds should know the associated toxic effects so that proper precautions are taken when handling them. Unless the great variability in the effects of beryllium and its compounds is appreciated, much confusion may arise when the literature is reviewed. The many factors which contribute to this variability should be kept in mind. The effects of beryllium on experimental animals are twofold: namely, the acute and the prolonged or chronic effects. There seems to be more uniformity among authors with respect to the acute effects than in respect to the chronic effects. Some of the factors that contribute to the variable results reported in the literature are the particle size, state of aggregation, temperature at which the beryllium was processed, solubility of the material used, chemical structure and physico-chemical properties, concentration, dosage and duration of exposure, method of administration, time between the administration and the observations, and the species of animal used in experimental observations.

* Ultrasound is a term used to describe those frequencies in the acoustic spectrum which are beyond the range of the human ear. The standards committee on electro-acoustics (a sub-committee of the American Standards Association) has set the lower limit of ultrasonic frequencies at about 15 kilocycles per second. Ultrasonic waves differ from the familiar audible waves only in frequency and, therefore, wavelength.
Many reports of the toxic effects of beryllium and its compounds have been published by research teams, clinicians and industrial administrators. A complete review is beyond the scope and purpose of this report, and only references pertinent to our particular study with general surveys of the literature will be included.

We are indebted to the late Dr Leroy U. Gardner, whose untimely death in 1946 occurred before he had completed his report. His interesting abstract with Heslington was presented to the Federation of American Societies for Experimental Biology in March of that year. He described for the first time osteogenic sarcoma produced in rabbits by the intravenous injections of zinc-beryllium-silicate and beryllium-oxide. He was the first, too, to recognise splenic atrophy in these animals. George W. Wright published an important paper prepared from Gardner’s original notes. Gardner’s investigations on bone tumours induced in rabbits by beryllium were confirmed by Hoagland, Grier and Hood (1950) and by Dutra and Largent (1950).

Hyslop and associates (1943) surveyed the literature and gave a comprehensive report of the toxic effects of beryllium as they were known at that time. It is unfortunate that the first sentence of their summary is the one most widely quoted: "The foregoing investigation indicates that beryllium is of itself not toxic." This conclusion was short-lived and it was not confirmed by later investigators.

Scientists at the University of Rochester School of Medicine and Dentistry (Hall et al. 1950, Stokinger et al. 1950, 1953) have studied the toxicity of beryllium. They have investigated the acute effects on various animals representative of the carnivora, herbivora, rodentia and primates. Thus far they have published studies on a total of eleven species, namely: cats, dogs, chickens, rats, rabbits, guinea-pigs, goats, monkeys, mice, pigs and hamsters. Contrary to the report of Hyslop and co-workers (1943) the group at the University of Rochester stated: "Not only is beryllium unquestionably a toxic agent but it is toxic in such small quantities as to be among the most toxic chemically of all elements yet investigated..." They found that quantities of the order of millimicrograms are toxic.

Gardner (1946) was curious as to the possibility of some other factor being a trigger-mechanism in association with beryllium, thus producing toxic effects. One such other factor has been shown to be fluorine by the investigators at the University of Rochester (Stokinger et al. 1953). They also studied deposition of beryllium in the tissues and its excretion.

Schubert and White (1950) also studied the distribution and excretion of beryllium in adult dogs with the radioactive isotope of beryllium, Be⁷. They reported that most of the retained Be⁷ was in the skeleton, particularly the long bones, 128 days after injection.

EXPERIMENTAL PROCEDURE

Ten healthy adult male rabbits were selected for our initial study. Five millilitres of a 1 per cent suspension of "zinc beryllium silicate"* was injected through an ear vein twice each week for ten weeks. Thus during that time each animal received 100 millilitres of the suspension, the equivalent of 1 gramme of the beryllium preparation. Since beryllium in the form of its oxide constituted 3-36 per cent of the suspensions injected, each animal received 33-6 milligrams of beryllium oxide.

Throughout the time of the injections and subsequent housing of the animals, the laboratory workers took the greatest care to guard against dangers of berylliosis which has been shown to develop after inhalation of the smallest amounts of this toxic element (experiments elsewhere have shown that beryllium is excreted very slowly through the kidneys). Gloves and masks were worn during the injections, and caretakers were cautioned to keep moist all the litter under the housing cages. Each animal was quartered individually in a

* A 10 per cent suspension of this beryllium preparation was supplied in generous amounts to us by Dr Arthur J. Vorwald, director of the Trudeau Foundation, Saranac Lake, New York, for which we are grateful.
large wire cage and maintained on a metal screen. They were given fresh water daily as well as a standard adequate laboratory ration of food.

After the injection period each animal was examined radiographically once a month until it was evident whether or not osteogenic tumours were developing. Some animals were killed during the early stages of tumour development, whereas others were permitted to progress to a later stage. At death each animal was examined carefully by necropsy. Bones which had shown radiographic evidence of tumour involvement, as well as certain others which did not present such evidence, were removed and fixed in 10 per cent formalin, decalcified and appropriately stained. The spleen, liver, adrenal glands, lungs and all other organs in which metastasis was suspected were removed, fixed and prepared for histological study. The stains used to show changes in the spleen were haematoxylin and eosin, Mallory-Heidenhain stain, and Gomori's silver-impregnation for reticulum. In some instances an iron stain was used for sections of the spleen. In others, the diphenylcarbazide reaction was employed to demonstrate the presence of beryllium in the tissues. Sections of the adrenal glands were stained with Sudan IV to demonstrate the extent to which lipide had been deposited in the cortices.

RESULTS

All the animals were apparently healthy throughout the experiment. They tolerated all the twenty injections well. They gained weight during the period after injection and were reasonably free of respiratory infection. Some of the animals with advanced tumour involvement showed signs of paralysis of the hind limbs. This may or may not have been related to the extent of the developing sarcomas.
In five of the ten rabbits osteogenic sarcomas were recognised radiographically nine to eleven months after the injection period, the diagnosis being confirmed in each instance by histological section. In the animals in which tumours developed we observed sclerosis in the medullary cavities of the long bones as early as three months after all injections had been discontinued (Figs. 1–3).

**Fig. 3**
Section showing new bone as well as early osteoblastic osteogenic sarcoma in the medullary cavity of the right humerus. (Hematoxylin and eosin; × 90.)

These osteogenic sarcomas showed a predilection for the metaphysial regions (Fig. 4). Multiple tumours often developed in the same animal. Metastasis to the lungs was not uncommon; and in one animal the lungs were completely infiltrated with a firm osteoid tumour which was obviously a metastatic growth from the primary sarcoma in the femur (Figs. 5 and 6).
The rate of tumour development varied from one animal to another, as might well be expected. The rate of growth was more rapid in some than in others; but the sequence of events, leading to the formation of the metastasising tumour, was similar in all five rabbits. The following is a typical report.

Within six months after all injections had ceased the first obvious change in this animal was the filling in of the medullary cavity of the long bones with a new bone matrix. This was visible in radiographs of the left tibia and right femur. Three months later (nine months after any beryllium had been given) an osteoblastic lesion appeared in the metaphysial region of the upper end of the left tibia, as well as an osteolytic lesion in the metaphysial region of the lower end of
FIG. 7
An osteoblastic lesion in the upper metaphysial region of the left tibia and an osteolytic lesion in the lower metaphysial region of the right femur.

FIG. 8
Chondroblastic sarcoma of the distal part of the right femur. (Hematoxylin and eosin; ×80.)
the right femur (Fig. 7). Microscopic sections from the tumour of the left tibia showed it to be a sclerosing osteogenic sarcoma; whereas sections from the tumour of the right femur showed it to be a chondroblastic sarcoma (Fig. 8). Histological sections prepared from parts of the right humerus which had not shown radiographic evidence of a tumour, showed early changes suggesting the development of an osteogenic sarcoma—there was new bone formation in the medullary spaces as well as fibrosis. In this animal there was also hepatic necrosis and in one part of the liver there was a clearly defined metastatic lesion with osteoid tissue.

Of all the changes observed in the animal just described, as well as in all others in which osteogenic sarcomas developed, the most significant were the marked atrophic changes in the spleen. Gardner and Heslington (1946) had noted splenic atrophy, and Hoagland, Grier and Hood (1950) stated that the spleen was smaller in animals with osteogenic sarcoma. Our studies had led us to believe that there is a definite correlation between splenic atrophy and the formation of a tumour. In all five animals which did not produce bone tumours the spleens were normal despite the fact that the animals had received as much beryllium as those which did produce tumours.

The changes in the spleen induced by beryllium, not only during the time of injection but also during the months leading up to the production of the tumours, were most interesting. A detailed report of this part of our study will be presented elsewhere; it suffices to state here that extreme atrophy occurred in all animals with osteogenic sarcoma—in fact, in some cases the atrophy was so great that all that remained of the spleen was an aplastic fibrotic cord of residual splenic tissue persisting in the gastro-splenic mesentery. Since the fibrotic spleen was largely devoid of circulating blood, the colour patterns had disappeared and the normal deep red was replaced by the white patches of an aplastic organ (Fig. 9). Small areas of functional splenic pulp remained; but the transition to the aplastic condition was obvious. Figure 10 is a section through part of a spleen in which complete atrophy had not yet occurred. The Malpighian corpuscles—the source of lymphocytes—and the reticulo-endothelial components persist; and the red pulp contains large, irregular, concentrated masses of material which the diphenylcarbazide reaction showed was beryllium. A section through the aplastic regions of this spleen (Fig. 11) shows the relatively complete loss of cellular structure. The persistent central artery, and the few remaining lymphocytes, indicate the residue of a Malpighian body. Scattered throughout are a few fibroblasts, a few reticular cells and circulating erythrocytes. When the Gomori stain was applied to these areas a dense, closely packed, reticular network, embracing all the splenic substance, was shown.

The sequence of events which occurs in these spleens as a result of the injections of beryllium is not unlike that arising from the injection of colloidal preparations or particulate materials. Local histiocytes of the sinusoids and the cords of Billroth first engulf the injected substance which is circulating through the red pulp. These cells increase in size, break down under the toxic influence of the element, and are then destroyed. The Malpighian corpuscles proliferate, increase in size, and release more lymphocytes which migrate into the red-pulp areas, there to transform into phagocytic histiocytes. These newly formed cells engulf the material, only again in their turn to die as a result of toxic reaction. Thus gradually the
cycle of lymphocytes, to phagocytosing histiocytes, to cell-destruction, proceeds until all Malpighian corpuscles are exhausted and the spleen becomes a non-functional aplastic organ, consisting largely of an acellular reticular network.

Fig. 10
A portion of the spleen in which complete atrophy had not yet occurred. (Hematoxylin and eosin; ×65.)

Fig. 11
Section of spleen with relatively complete loss of cellularity. (Hematoxylin and eosin; ×280.)

COMMENT
In this study we have confirmed the observations of previous workers with beryllium, namely that osteogenic sarcomas will develop after a considerable time in some of the animals injected with preparations containing it. Gardner and Heslington (1946) produced tumours
in seven of twenty-four rabbits. Hoagland, Grier and Hood (1950) produced them also in seven of their twenty-four animals; and Dutra and Largent (1950) produced them in six of nine rabbits. We produced osteogenic sarcoma in five of our ten animals. The authors quoted did recognise such splenic changes as reduced size or even atrophy, but little consideration has hitherto been given to a potential relationship between such atrophic or aplastic changes in the spleen and the development of tumours in the bones. Although there is not yet any proof of such a relationship it is notable that osteogenic sarcoma developed in the animals that had an aplastic and obviously non-functional spleen. We propose to explore these relationships in further studies, testing the influences that splenic substances may exert upon the course of induced bone tumours. We are now determining the incidence of beryllium-induced bone tumours in animals which underwent splenectomy before they were given injections of beryllium. If functional splenic tissue does exert an inhibitory influence upon tumour-genesis in the bones, then in such animals from which the spleen has been removed an increased incidence of sarcoma may well be demonstrated.

As a corollary to this concept of an interrelationship between the spleen and bone tumours, we have begun with Dr Warren Bennett of this Clinic a study of the state of the spleen in patients who died of osteogenic sarcoma.

Our original purpose, namely the production by chemical means of osteogenic sarcomas that would serve as test objects suitable for the application of ultrasonic energy, is now being carried out, and the effects of ultrasound on such tumours are still being studied.

SUMMARY AND CONCLUSIONS

The method of producing osteogenic sarcoma in rabbits by the injection of beryllium in the form of "zinc beryllium silicate" is presented. In five of ten animals which had such injections, osteogenic sarcomas developed several months later. There was new bone formation in the medullary cavities of the long bones before malignant changes were apparent. It is of particular interest to note that there was atrophy of the spleen in those animals in which bone tumours developed, whereas the spleen seemed to be quite normal in the rabbits which did not develop bone tumours. The tumours usually developed in the metaphysial regions. More than one tumour often developed in the same animal.

We are indebted to Dr Arthur J. Vorwald, Director of the Trudeau Foundation, for his interest and suggestions concerning the administration of beryllium; and to Dr Friedrich Klemperer of the Saranac Laboratories, Dr Frank R. Dutra of the Kettering Laboratory at Cincinnati, Dr Harriet L. Hardy of the Occupational Medical Service, Cambridge, Massachusetts, and Dr Charles L. Dunham of the Atomic Energy Commission for their advice and suggestions during the early preparations for our study.

We also wish to thank Dr David Dahlin of the Section of Surgical Pathology of the Mayo Clinic for reviewing the histological preparations and confirming the presence of osteogenic sarcoma; Mr Robert Schmit, chief technician of the Section of Pathologic Anatomy, for his help in the preparation of materials for histological study and for identifying beryllium in the tissues; and Mr Glenn Christensen, Mr Sam Amundson, Mr Howard Hanson, Miss Betty Hennessey and Miss Agnes Conger for their technical assistance.

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