periments also indicate that maximum excretion of salicylate by the kidney will occur when an elevation of body fluid pH and urine pH are accompanied by a marked diuresis. THAM, which promotes these changes, may be useful in treatment of salicylate poisoning in man.

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Effect of Internally Deposited Radioisotopes upon Blood Vessels of Cortical Bones.* (26108)

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Recent reviews by investigators dealing with internally deposited radioisotopes in bone state there is no evidence that injury to the bone was secondary to injury to small blood vessels directly supplying the bone(1, 2). They conclude that injury to the various cellular constituents of the bone appears to be a direct effect of the radiation upon them. This conclusion is based on the study of bones from radium patients in which activity is found throughout bone, and the alpha particles appear to be killing osteocytes directly.

In the present study we demonstrate that much of the damage in cortical bone is due to vascular occlusion. These studies have utilized dogs containing radium, plutonium or radiothorium in which a new technic of vascular injection of India ink-gelatin has been used to demonstrate the disturbance in blood supply. Unlike radium, which is distributed throughout bone, plutonium and radiothorium are localized on bone surfaces and the short range of the alpha particles located about haversian canals allows the major portion of the haversian systems and all the interstitial lamellae to be free of activity. Thus, if massive devitalization of bone is observed, it cannot be related to direct killing by alpha rays.

Materials and methods. The materials used were the mid-diaphyses of tibiae from 4 normal and 23 beagles that died or were sacrificed from 1 to 1724 days following administration of radium-226, plutonium-239 and

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TABLE I. Summary of Experimental Dogs.

Parent isotopes	Dose (μc/kg)	No. of animals
Plutonium-239	3.0	18
Radium-226	10.0	3
Radiothorium-228	.9	3
Control	—	6

radiothorium-228 (Table I). Seven of these animals were part of the Utah Beagle chronic toxicity studies of bone-seeking radioelements at the Radiobiology Laboratory, Univ. of Utah College of Medicine. Augmenting the chronic toxicity dogs were 15 plutonium dogs and 1 radium dog which were serially sacrificed to give information regarding distribution of the radioelements and sequence of histopathologic changes.

Young adult Beagles of mixed sexes between 14 and 24 months were given a single intravenous injection of one of the principally alpha emitting bone-seeking radioisotopes (plutonium, radium or radiothorium). Injection solutions were prepared in citric acid-sodium citrate buffer, 0.08 M in total citrate and pH 3.5(3). The chronic toxicity dogs were permitted to live until moribund. All dogs were anesthetized with sodium pentothal, each given 100 mg of heparin intravenously, and 15 minutes later, exsanguinated. A mixture consisting of Higgin's India ink, 1 part, and 14% solution of calfskin gelatin, 1 part, was injected (at 37°C) into the femoral artery of a disarticulated hindlimb. After injection of 400 ml of fluid, the limb was chilled (–20°C) to solidify the gelatin. The bones were then disarticulated, defleshed and radiographed. For this study adjacent 2 cm long specimens of the mid-diaphysis of the tibia and whole metatarsi were sawed and fixed in Zenker-formol and absolute acetone. Specimens fixed in Zenker-formol were embedded in celloidin, sectioned and stained with hematoxylin and eosin to demonstrate ink filled vessels and viable osteocytes (Fig. 4). Details of vascular injection as well as celloidin technics have been reported(4).

Specimens fixed in absolute acetone were used in preparing 50-80 μ thick transverse ground sections. The bone was first sawed to 200 μ in thickness with a special rotary saw. The sawed sections were then ground to the desired thickness between 2 ground glass plates using levigated alumina suspended in absolute alcohol. Water was avoided to insure against leaching of the isotopes. These sections were microradiographed on Lippmann film‡ with 10-Kv x-rays. Absorption of the x-rays in the section was such as to demonstrate different degrees of mineralization. Following the microradiograph, an autoradiogram of each section was obtained by exposing the sections on Eastman Kodak's NTB, 10 μ thick, "t" coat nuclear track plate for 30 to 50 days. The plates were developed in Kodak's D-19 for 4 minutes, fixed in acid fixer for 10 minutes, rinsed in tap water for one hour and allowed to dry in air.

Results. Autoradiographic findings. Two contact autoradiograms of a region of a transverse section of tibiae containing either plutonium or radium are presented to exemplify representative localization patterns. When plutonium or radiothorium was taken up by cortical bone, radioactivity was deposited on bone surfaces in varying concentrations depending on location. There was an intense concentration on the endosteal surfaces, a moderate concentration on periosteal surfaces, and on surfaces of resorption cavities and vascular channels, and a much lower concentration about haversian canals (Fig. 1). It should be emphasized that the range of the alpha particles of plutonium and radiothorium have short range in bone (about 24 μ), which meant that only a portion of the osteone about the canal is irradiated directly by alpha particles, while the remainder of the osteone and the surrounding interstitial lamellae were free of activity.

As the result of internal reconstruction, some of the initial deposition of plutonium or radiothorium on bone surfaces are absorbed into the blood stream. Subsequently, some of the plutonium or radiothorium then is redeposited as a lighter, diffuse pattern in the new generations of osteones.

When radium was taken up by cortical bone, the activity deposited throughout bone

‡ Obtained from Gavaert Co. of America, Inc., Los Angeles, Calif.

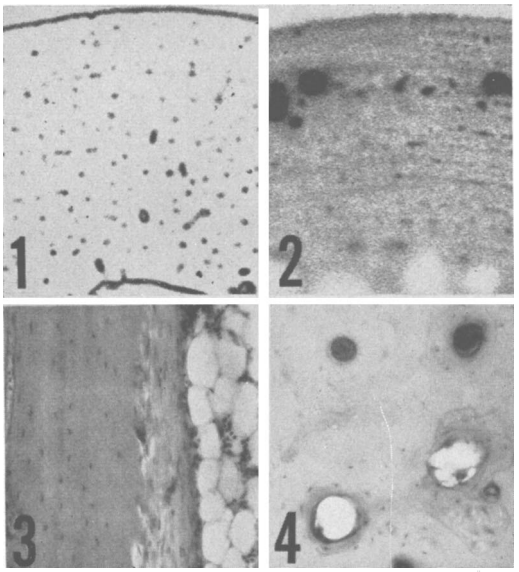

FIG. 1. A contact autoradiogram of a portion of transverse section of tibial diaphysis to show localization of plutonium on bone surfaces (endosteal, periosteal, haversian canals and vascular channels). Note large fraction of bone free of radioactivity (× 13).

FIG. 2. A contact autoradiogram of cortical bone containing radium to show intense deposits of radioactivity ("hotspots"), low "diffuse" deposition throughout remainder of bone and many punctate areas of moderate radioactivity (× 13).

FIG. 3. Endosteal surface of radius containing plutonium showing active bone resorption under the intense fibrosis. Celloidin section, H & E (× 68).

FIG. 4. A cross section of metatarsus containing plutonium and inj. with India ink-gelatin at autopsy to show osteones with India ink in haversian canal and viable osteocytes (upper right) and osteones with plugged canals and empty lacunae (upper left). Note much of the interstitial lamellae are with empty lacunae. Celloidin section, H & E (× 68).

both in areas of bone growth and in bone existing at time of administration of radium. The regions of bone growth were characterized by intense deposits of radium commonly called "hotspots," while the remainder of the bone exhibited a much less intense deposit called "diffuse" activity (Fig. 2). Also many punctate regions of moderate deposition were found about haversian canals.

Sequence of histopathologic changes in cortical bone containing plutonium. The earliest changes occurred at the endosteal surfaces of the compacta where initially there was a heavy concentration of plutonium. This resulted in destruction of endosteal cells within one week, followed by loss of osteocytes located within the range of the alpha particles deposited on the endosteal surfaces. In turn, this was followed by formation of a dense connective tissue (fibrosis) at 2 months and occurrence of abnormal bone resorption beneath the fibrosis at 3 months (Fig. 3).

Later changes observed in the bulk of the cortical bone, which contained principally a light concentration of plutonium about haversian canals, involved first the plugging of many haversian canals at 400 days post injection. The proof that the canals were functionally obstructed and not simply artifacts was supplied by sections of tibiae and metatarsi injected with India ink-gelatin. Fig. 12 exemplified a case of a dog containing radiothorium which showed a plugged vessel in a longitudinal section of metatarsus, and only India ink was found pooled on the periosteal surface and in the marrow cavity, in contrast to rich network of vessels filled with India ink in bones of control animals (Fig. 11). To date, the only observations of the character of the haversian canal plugs were that the majority have slightly basophilic staining properties and were not calcified, while a few plugs were of hypermineralized material.

Although approximately half of the canals were plugged at 400 days, the majority of the osteocytes appeared histologically normal, while only a few appeared shrunken. However, increasing burden time resulted in increased plugging of canals which in turn limited fluid circulation sufficiently to result in the death of osteocytes in affected osteones and adjacent interstitial lamellae. It should be emphasized again that the majority of these dead osteocytes were in regions free of activity. All the osteocystes within osteones having a blood supply were viable. The direct relationship of blood supply (India ink filled vessels) and viable osteocyte was apparent at 777 days after injection of plutonium (Fig. 4).

The fact that not all haversian canals were plugged allowed some internal reconstruction to occur; thus, new osteones were formed. However, the process of reconstruction at 2½ years in these animals was abnormal, re-

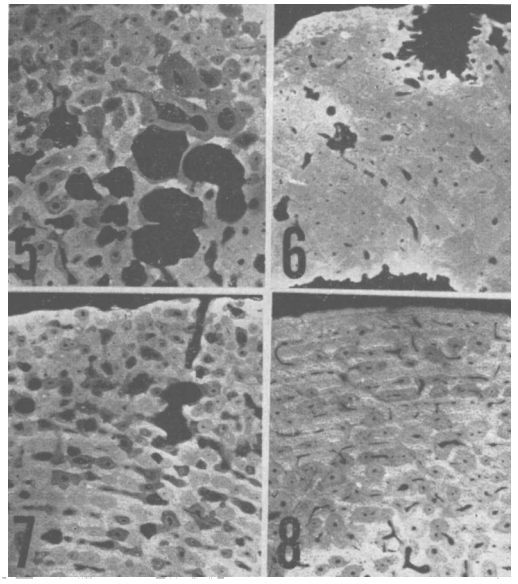

FIG. 5-8. Microradiograms of cross section of the tibial diaphysis (× 13).

FIG. 5. Compacta of dog given 10 μc/kg of radium. Note large resorption cavities, hypocalcified osteones and hypercalcified haversian canal plugs. High calcification appears white on the photograph.

FIG. 6. Compacta of dog given 0.9 μc/kg of radiothorium. Note large area of periosteal resorption, ragged endosteal surface, few small cavities and bland mineralization.

FIG. 7. Compacta of dog given 2.5 μc/kg of plutonium. Note occasional large cavities, numerous hypocalcified osteones and a few hypercalcified plugs.

FIG. 8. Compacta of control dog. Note that osteones are slightly less mineralized than interstitial lamellae.

sulting in formation of many large resorption cavities and abnormal osteones. In general, resorption cavities were slightly larger than average, irregular in shape and often filled with fibrous tissue. A few cavities were 4 to 6 times the size of a normal resorption cavity (Fig. 7 and 10). The abnormal osteones were generally structurally disorganized and abnormally calcified (Fig. 5, 7, 9 and 10).

No spontaneous fractures occurred in long bones of dogs given 2.5 μc kg of plutonium in contrast to the numerous incidence of fractures in long bones of dogs given 10 μc/kg of radium and 0.9 μc/kg of radiothorium. Nevertheless, 2 animals given 0.9 and 0.27 μc/kg of plutonium developed microscopic osteogenic sarcoma arising from cortical bone.

Changes in cortical bones containing radium. Although only a few serial sacrificed dogs were studied, the compacta in animals given radium demonstrated similar changes. Besides the varieties of abnormal osteones and regions of necrotic bone observed in dogs given plutonium, the bones with radium contained unusually large cavities (Fig. 5). The portion of the compacta that was necrotic (loss of osteocytes) represented approximately 60-85% of the bone. These bones also differed in that numerous spontaneous fractures of the long bones were observed in animals given 10 μc/kg of radium.

Changes in cortical bone containing radiothorium. In contrast to radium and plutonium, concentration of activity about haversian canals in animals injected with 0.9 μc/kg of radiothorium resulted in the almost

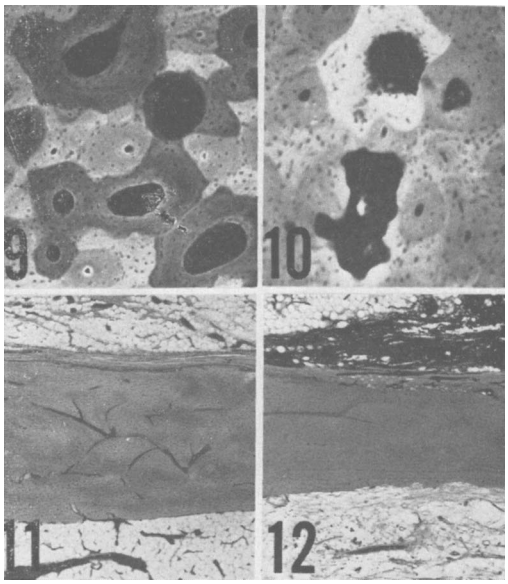

FIG. 9. Microradiograph of a cluster of hypocalcified osteones with enlarged haversian canals (× 68).

FIG. 10. Microradiograph showing an abnormally high mineralized osteone (white structure) and an adjacent irregular shaped resorption cavity (× 68).

FIG. 11. Longitudinal section of a metatarsus inj. with India ink from a control dog. Note network of vessels filled with ink in the compacta, marrow and juxtaperiosteally. Celloidin section, H & E (× 13).

FIG. 12. Longitudinal section of a metatarsus inj. with ink from a dog given radiothorium. Note the single plugged vessel in compacta without ink, endosteal fibrosis, meager supply of ink filled vessels in the marrow and pooled ink on the periosteal surface. Celloidin section, H & E (× 13).

complete shut down of circulation and total necrosis of the compacta. This was best exemplified in the metatarsus whose vascular tree was injected with India ink in which the compacta was free of vessels containing India ink. The ink was located in meager amounts in the marrow and pooled on the periosteal surface. The region of pooled ink corresponded to the region of resorbed bone (Fig. 6 and 12). The few irregular resorption cavities shown in the microradiograph in Fig. 6 were formed early after the acquisition of radiothorium before the loss of total vascularity resulting in total inhibition of internal reconstruction. The bones illustrated in Fig. 6 and 12 were pre-injection bones, which had subsequently lost their viability (dead bone) as the result of loss of vascularity. The staining characteristics of non-viable bones were very poor and appeared quite uniform in mineralization in that osteones were only slightly less mineralized than interstitial lamellae (Fig. 6) as compared to the normal (Fig. 8).

Another constant finding was the occurrence of spontaneous fractures of long bones in every dog given 0.9 μc/kg of radiothorium.

Discussion. Injury to blood vessels has been regarded as a feature of irradiation lesions by many investigators who have given external irradiation, radium and polonium to animals and patients(5,6,7). However, there is no direct evidence that this is a major factor in causing damage to bone. Rowland, *et al.*(8) demonstrated plugged haversian canals and stated that this "implies the existence of widespread circulatory damage throughout bone." Our study shows by direct observation and a vascular injection technic that there is extensive functional vascular obstruction which results in bone necrosis.

The nature of the thrombosed haversian canals differs considerably from the changes in vascular tissue in other organs receiving irradiation(5,6,7). We have observed the apposition of mineralized matrix or secretion of a "matrix-like" material by the cells in the haversian canals. This may be simply the elaboration of mucopolysaccharide by vascular endothelium as shown by Curran(9) or of polysaccharide material observed in blood vessels after acute irradiation by Andersen (10). In our laboratory, plugging of haversian canals also has been observed to occur in the normal aging process in dogs.

The abnormal character of the osteones and the existence of large resorption cavities have been described in earlier reports dealing with radium patients(8,11,12,14). The abnormal calcification in form of hypercalcified rings has been seen in bones of older people (13) and Beagles. However, the character and scattered distribution of the large cavities in cortical bone induced by alpha emitters differs from the cavities seen in osteoporotic humans.

It is generally accepted that osteocytes can revert back into osteoblasts and function to lay down new bone. However, in the compacta of these animals much of the bone lacks osteocytes, which would mean that the blood vessels must bring in fibroblasts into the area to form osteoclasts and osteoblasts to participate in bone remodelling. If the aberrant cell is the fibroblast then it would be very difficult to relate this cell with direct alpha ray damage due to the fact that nearly all activity is located in the existing bone. The peculiar action of the osteoblasts could be the result of a disturbed local environment such as altered vascular supply. Nevertheless, direct injury by alpha rays must not be overlooked in that there is always a small amount of activity circulating in blood.

We note that the large cavities in cortical bone containing radium contained either fibrous connective tissue or bone marrow cells. It is believed that one of the factors concerned with formation of large cavities is increased resorption due to enhanced devitalization of bone at areas of intense irradiation ("hotspots"). This is supported by observation in which we relate devitalized region of bone to "hotspots" in high resolution autoradiography studies in our laboratory. In the region of "diffuse" deposits of radium the osteocytes are viable. Often the intensity of the irradiation at "hotspots" is too damaging to allow bone remodelling to be carried out. It is logical to speculate that the continual presence of the low intensity

"diffuse" deposits of radium in bone may be sufficiently injurious to inhibit the apposition of bone, but allow for regeneration of circulating bone marrow cells and fibrous connective tissue.

Occurrence of spontaneous fractures in long bones of dogs given 10 μc/kg of radium and 0.9 μc/kg of radiothorium should be considered. Spontaneous fractures are quite common in radium patients(1,11,14). In our study it is believed that the events which weaken the bones containing radiothorium are principally due to total devitalization and increased mineralization of cortical bone aided slightly by resorption of endosteal and periosteal surfaces. In the cases of bones containing radium, the weakening can be attributed partially to devitalization of bone and partially to the large cavities (diffuse osteoporosis). Of course, the adverse effects of alpha emitters on collagen and bone matrix, whatever they may be, must not be overlooked.

Summary. It has been observed that after an extensive latent period following acquisition of plutonium, radiothorium and radium in cortical bone, plugging of the haversian canals occurs which in turn results in bone necrosis. Investigation of this phenomenon with a vascular injection technic and a bone-seeking alpha emitter (plutonium) which deposits on surfaces of haversian canals and allows the remainder of the osteones and adjacent interstitial lamellae to be free of activity, show that death of osteocytes is principally the result of disrupted blood supply rather than direct alpha ray killing. With radiothorium, concentration of alpha particles about haversian canals was sufficient to result in total plugging of the vascular tree within the haversian canals resulting in complete bone necrosis and inhibition of internal reconstruction of cortical bone. With radium and plutonium only about half of the canals are plugged, which allows internal reconstruction to proceed; however, the nature of the remodelling in this environment is abnormal (formation of large cavities and abnormal osteones). It is also suggested that both devitalization of bone and occurrence of large cavities weakens the bone which in turn results in spontaneous fracturing of long bones.

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