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INVESTIGATIONS OF ^{90}Sr IN DOGS

I. Pathogenesis of radiation-induced bone tumors

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Abstract

Purebred beagle dogs given ^{90}Sr and unirradiated controls were studied for over two decades. Pregnant females were fed different doses of ^{90}Sr from day 21 post-conception until the offsprings reached an age of 540 days. In an additional experiment two dose levels were given in a single intravenous dose to dogs 540 days old. Radiographically the earliest skeleton lesions were characterised by small linear, solitary, cortical lucencies. These as well as tumors were more frequently noted in the higher exposure levels. They affected the appendicular skeleton almost as frequently as the axial skeleton. The lesions were predominantly found in the diaphysis, at the angle and near the acetabulum in the tubular bones, mandible and pelvis, respectively. The lesions within the diaphysis originated in the cortical bone. Histologically these lesions were characterised by different types of porosities. These could be empty or filled by a defect and/or immature, dysplastic fibrous repair tissue, within the frame of which malignant transformations seemed to take place as evidenced by malignant clones and microosteosarcomas. A comparison is made of the histologic events in dogs and mice and a tentative pathogenesis of ^{90}Sr induced bone tumors is discussed.

For the study of radionuclide-induced neoplasia, systematic investigations of the pathogenesis of precancerous and early stages of malignancies taking place during the latent period seem extremely important for a better understanding of the radiation cancerogenesis. Nonetheless, such investigations appear to be rather neglected in this field of research.

Early phases of ^{90}Sr-cancerogenesis have been studied in mice (18, 19, 24, 26, 29, 33, 40). Similar work in mice on ^{226}Ra has been done by GOSSNER et coll. (10) and LUZ (20). The effects of radiostrontium on bones was studied in rats by LITVINOV (15–17) and in rabbits by OWEN & VAUGHAN (34). In dogs, ^{226}Ra-induced lesions were observed by JEE (12) and by POOL et coll. (36). Bones from radium exposed people have been studied by AUB et coll. (1), among others. Specially designed comparative interspecies investigations are few and seem mainly to have been performed by POOL et coll. (36).

The material that forms the basis for this investigation has been obtained from dogs fed or injected with varied quantities of ^{90}Sr. These animals were observed for entire life spans and were generally killed when death was imminent. While a serial killing of the ^{90}Sr-exposed dogs would have been ideal for the characterization of the different pathogenic events preceding the development of radiation-induced tumors, such material was not available because of ethical and economical reasons. The present material, in spite of its limitations, offers a good opportunity to develop a better understanding of radiation-induced tumorigenesis of the dog. The suitability of material collected at death is emphasized by tissues of several dogs that showed a spectrum of bone lesions representing bone disturbances

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Table 1

Identifying treatment codes, quantities of ^{90}Sr administered, cumulative skeletal doses of beagle dogs fed or injected with the radionuclide. The number of tumors exceeds the number of dogs dying because some animals had multiple tumors. In addition, not all tumors were visible radiographically

Treatment code	Nominal quantity administered[1] (MBq)	Mean±SD Skeletal dose (Gy)	No. of dogs dying of bone cancer[2]	No. of bone tumors observed	
				Radio-graphically	Histo-logically
Sr-fed[3]					
D00	0	–	0/80	0	0
D05	0.5	0.3±0.1	0/78	0	0
D10	1.5	1.3±0.3	1/40	0	1
D20	8.9	8.0±2.1	0/66	0	0
D30	27	26±7.1	2/65	2	2
D40	80	61±20	6/61	5	5
D50	240	97±42	15/60	11	21
D60	720	130±38	10/19	19	20
Sr-injected[4]					
S20	1.4	7.5±3.2	1/20	1	3
S40	12	62±20	6/26	6	5

[1] Assuming a 'typical' 10-kg beagle.
[2] From Book et coll. (5).
[3] Feeding of ^{90}Sr began 21 days post-conception, continued through pregnancy and lactation, and, was completed at 540 days of age.
[4] Injection of ^{90}Sr was via a single intravenous injection of $^{90}SrCl_2$ at 540 days of age.

seen among dogs of many different ages, with varying types of bone destruction, repair, and tumor development. A careful analysis of the histopathology of these lesions presents an approximate indication of the type of osteodysplasia that is most likely to be associated with tumor development.

After we present the results of this analysis, the most pertinent lesions found in the dogs will be compared with those found in mice treated with single intraperitoneal injections of ^{90}Sr (24, 26). Of particular interest in this respect is the evaluation of differences and similarities of bone tumors of the two species with regard to the type of tumors and their micro- and macro-localization, and the influence of radiation dose and other factors (e.g., sex) on their pathogenesis.

Methods

Bone tumors described in this report were produced in beagle dogs given long-term exposure to ^{90}Sr. The dogs, purebred beagles from the outbred, closed colony at the Laboratory for Energy-Related Health Research of the University of California at Davis, are part of an experimental population of animals exposed to ^{90}Sr and ^{226}Ra and unirradiated controls. They have been under study for well over two decades in a program that seeks to describe the comparative long-term toxicity, of low-level exposures of the two bone-seeking radionuclides and the relationship of radiation dose, biologic effect, and time. Among the goals of that large experiment is the prediction of effects of internal emitters for exposed human populations. Even though that study is yet to be completed, the rationale, experimental design, methods, and some of the results have been presented (4, 5, 37, 38).

Most of the tumors described in this paper arose in dogs fed a ^{90}Sr-containing diet. A few tumors were in dogs injected with ^{90}Sr. No bone tumors have been produced in our unirradiated control dogs.

Feeding of ^{90}Sr began (to animals designated as 'D') when a diet containing one of several concentrations of ^{90}Sr, as $SrCl_2$, maintained as a constant relative to the level of dietary calcium (Table 1), was

Table 2

Radiologically evident radiation induced bone tumors (^{90}Sr)

Tumor location	Level of exposure						
	D60	D50	D40	D30	S40	S20	
Appendicular skeleton							
Scapula	1		1				
Humerus							
Proximal			1		1		
Distal	6						
Radius							
Proximal	1						
Ulna							
Proximal		1					
Midshaft	1						
Femur							
Proximal	2		1				
Midshaft	1	1			1		
Distal					1		
Tibia							
Midshaft	1						
Axial skeleton							
Rib				1			
Skull	1	1					
Mandible	1	4	1				
Pelvis	2	3			1		
Vertebra							
Cervical	2				1		
Thoracic		1		1		1	
Lumbar			1				
Coccygeal					1		
Total	19	11	5	2	6	1	n=44

given to pregnant bitches beginning on the twenty-first day of gestation. That time corresponds to the approximate time of the beginning of skeletal development in the canine fetus. Feeding of the same concentration of ^{90}Sr in food continued throughout gestation and lactation, and at weaning, pups continued to receive the same food until 540 days of age, when skeletal development was complete. This treatment method was considered to result in a skeleton that was relatively uniform in its distribution of ^{90}Sr.

A smaller number of dogs (designated as 'S') were injected intravenously with one of two levels of ^{90}Sr in 0.1 N hydrochloric acid in saline (Table 1). The injection was given at 540 days of age. The distribution of skeletal radioactivity, hence, resulting from this treatment, would be relatively non-uniform.

All dogs were housed indoors to prevent environmental contamination during the administration of radioactivity. When administration of ^{90}Sr was complete, and after allowing some time for the elimination of radioactivity not deposited in the skeleton, animals were moved to outside pens where they remained for the duration of life. Medical treatment was provided as required.

Throughout the study, the dogs received periodic physical examinations, whole-body counting, and veterinary care, as needed. Dogs were subjected to whole body radiography at yearly intervals plus additional radiography as determined by clinical signs. Bone lesions of a destructive or productive nature were given special attention following their identification.

The whole-body counting, basic to the calculation of radiation dose, provided the means to determine the skeletal burdens of ^{90}Sr and its decay product ^{90}Y, assumed to be in equilibrium, by total-body bremsstrahlung counting with a 10 cm × 20 cm NaI (T1) detector (later, two detectors were used), in conjunction with a pulse-height analyzer (8). The bremsstrahlung spectrum was summed and compared with a frozen standard with a known ^{90}Sr-^{90}Y burden. A terminal count for each dog was made at death or just before euthanasia for dogs that were moribund.

The skeletal dose was calculated by standard dosimetric equations, utilizing the skeletal ^{90}Sr-^{90}Y burden, an estimation of skeletal weight, and time to give an expression of microcurie-days per gram of skeleton. That value, when combined with appropriate radiobiologic constants, allows the skeletal dose rate (in Gy/day) and the cumulative skeletal dose (in Gy) to be calculated, as discussed in detail by RAABE et coll. (38).

The tumors discussed in the following were primarily bone tumors that resulted in death or euthanasia of the affected dogs. After death, each dog was subject to a complete necropsy and to complete histopathologic examination. The results of all aspects of the study will be presented in future publications, at the completion of the experiment.

Results

Radiography. Bone tumors were identifiable radiographically in 31 dogs at the time of death or euthanasia. The total number of bone tumors in these dogs was 44 (Table 1).

The level of exposure of ^{90}Sr and the location of the bone tumor was noted (Table 2). Most tumors were noted in the higher exposure levels (D60, D50) and affected bones of the appendicular skeleton almost as frequently as bones in the axial skeleton. Within the long tubular bones, the lesions were predominantly found within the diaphysis. Within the mandible, one-half of the lesions was found at the angle. Within the pelvis, most lesions were near the acetabulum.

The appearance of the first radiographic signs of bone disease was dependent on the dosage and manner of delivery of the ^{90}Sr. All of the highest level (D60) dogs had radiographic changes in the skeleton by 3 years of age and the majority of tumors (13/19) had early destructive changes by 2 years of age. Bone lesions that developed into tumors in the D50 dogs were first detected radiographically between 6 and 9 years of age, while the premalignant bone lesions in the midlevel D40, D30, S40, and S20 dogs were first detected radiographically between 10 and 15 years of age.

The most common pattern noted radiographically was found first as a diaphyseal lesion and appeared to originate within cortical bone. This type of lesion was noted in 15 bones from 11 dogs (5 female and 6 male) and was only noted in those dogs in which the radioactive strontium was fed and in only the highest, most toxic levels (D40, D50, D60). The lesions were divided histologically as follows: 2 osteosarcoma, 6 osteoblastic osteosarcoma, 5 fibroblastic osteosarcoma, 1 mixed osteosarcoma, and 1 hemangiosarcoma. The lesions were distributed as follows: 6 humerus, 1 radius, 1 ulna, 5 femur, 1 tibia, 1 ileum.

This pattern was characterized first by the presence of small linear cortical lucencies. Usually they were solitary, but could appear as multiple lesions that tended to coalesce with the passage of time. The expansion of the lucent area usually occurred without stimulating either periosteal or endosteal new bone. This often resulted in a thin border of bone tissue surrounding the lesion. Usually no reactive bone was noted in the bordering bone tissue and the interface between lucency and surrounding bone was sharp and distinct. Rarely some repair was characterized by the development of sclerosis within the medullary cavity or a thin border of sclerotic bone around the lucent zones.

The expansion of the lesions was easily noted on sequential radiographs (Fig. 1). At this stage, however, the lesions did not cause clinical signs.

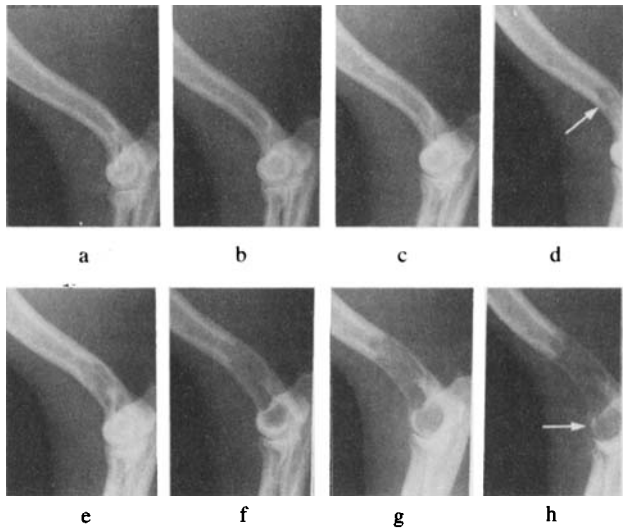
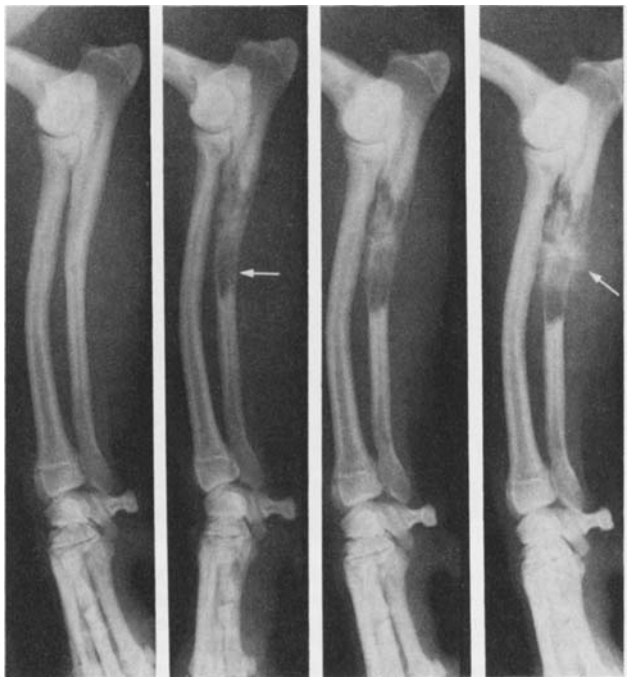


Fig. 1. Lateral radiographs of the left humerus of a male beagle dog (D60M30) with a progressively destructive cortical lesion that was diagnosed histologically as a hemangiosarcoma. The lesion was first clearly seen (d) and followed as it progressed proximally and distally into the humeral condyles (h) at which time the dog was killed. The slow progression of the lesion, the absence of any periosteal response, and the maintenance of a sharp border between tumor and surrounding healthy bone tissue is typical of many radiation (^{90}Sr) induced tumors. a) 206 days, b) 277 days, c) 366 days, d) 562 days, e) 650 days, f) 699 days. g) 732 days, h) 755 days.

In other cases, eventually the zone of destruction became large enough to weaken the bone sufficiently and stimulate a smooth periosteal bony response. This was linear in orientation and appeared to provide added strength for the weakened bone shaft. The zone of transition between the lesion and surrounding bone remained short and a sharp border to the lesion was maintained. Other lesions developed pathologic fractures with indistinct woven bone laid down over the fracture site. Thus, they had the appearance of a fracture callous around an unstable fracture (Fig. 2).

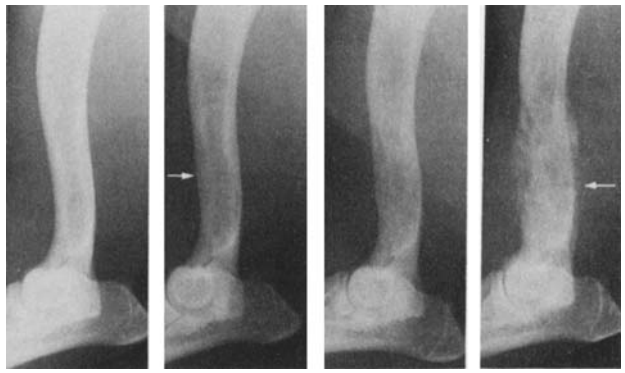
A second type of lesion progression was characterized by periosteal bony response with an orientation perpendicular to the shaft of the bone and a lengthening of the previously noted short zone of transition (Fig. 3). This new radiographic pattern was thought to represent malignant conversion of a previously benign lesion. Undetected pathologic fracture without fragment distraction could be considered as possibly contributing to this new bone formation.

The time required for these patterns to develop ranged from 29 to 416 days (median 140 days). While the rate of change was slow and uniform in



a b c d

Fig. 2. Lateral radiographs of the right front leg of a male beagle dog (D60M37) with a progressively destructive lesion that was diagnosed histologically as an osteoblastic osteosarcoma. The first change is a massive destructive lesion without periosteal response that maintains a distinct border with surrounding healthy bone (b). There is minimal progression of the lesion until a pathologic fracture develops with indistinct new bone response (d). a) 732 days, b) 887 days, c) 901 days, d) 915 days.



a b c d

Fig. 3. Lateral radiographs of the left elbow of a male beagle dog (D60M40) with a small zone of cortical lucency (b) that underwent malignant transformation with expansion in the shaft and production on an aggressive periosteal response (d). The lesion was diagnosed histologically as an osteoblastic osteosarcoma. The failure to preserve a short zone of transition between lesion and healthy bone plus the pattern of new bone production is typical of spontaneously occurring bone tumors although the time frame in which the changes are occurring is too long. a) 560 days, b) 650 days, c) 712 days, d) 771 days.

the early stages of the disease, it became much more rapid towards the end. Other lesions showed some repair of bone with an increased density and failed to develop into the more radiographically obvious malignant pattern. Similar cortical lucencies were seen in adjacent bones from the D60 group.

A slightly different pattern was noted in mandibular tumors in 3 male dogs in the D50 and D60 levels. The lesions were identified by a lucent pattern that started within the ventral mandibular cortex near the mandibular angle. Usually the lesions remained very quiescent in appearance. In one case, however, the lesion acquired a very malignant appearance with the formation of reactive bone and lost the sharp lesion border and the short zone of transition. The three lesions were described histologically as an osteosarcoma, a fibroblastic osteosarcoma, and an osteoblastic osteosarcoma. The lesions were followed radiographically for 0, 170 and 616 days from first detection until euthanasia.

Another pattern of tumor development was noted in the pelvis, with the lesions regularly appearing near the acetabulum in 5 female dogs. This tumor was more highly destructive than others. Early on, it caused cortical destruction as well as a pattern of periosteal new bone that could be either regular or irregular. These radiographic patterns suggested malignancy and made these tumors appear radiographically much more like spontaneous bone tumors (22). The lesions were followed for 0, 140, 176 and 245 days from first radiographic detection. In all cases the pelvic tumors were the cause of euthanasia. It was not possible to ascertain whether these tumors originated in cortical or cancellous bone. The dogs were from the higher groups (D50, D60 and S40). Four of the tumors were evaluated as osteoblastic osteosarcomas and one was a fibroblastic osteosarcoma.

In these pelvic tumors, as in the diaphyseal cortical tumors, there could be evidence of bone repair. This was especially noted in one case with a marked increase in bone density during the progression of the lesion.

Of 5 tumors in the vertebrae 4 appeared within the cancellous bone of the vertebral body and one within the cortical bone of the lamina. Two other tumors involving C1 could not be adequately evaluated radiographically. The vertebral tumors began as lucent areas, poorly defined within the body or lamina. If the body was involved, a lucent pattern lead to endplate destruction, disc space collapse, and

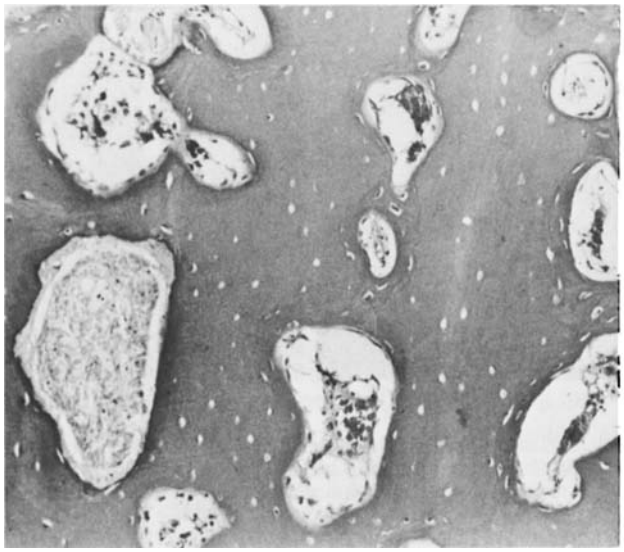


Fig. 4. Cross section, mid-diaphysis humerus from a ^{90}Sr fed dog (D50), dead at an age of 8.4 years. Empty osteocyte lacunae and/or atrophic osteocytes. Multiple, isolated cavities originating from osteons. Some cavities almost completely empty, one containing necrotic debris and a few showing apposition of bone. Mean skeletal dose 15.62 Gy. H-E ×125.

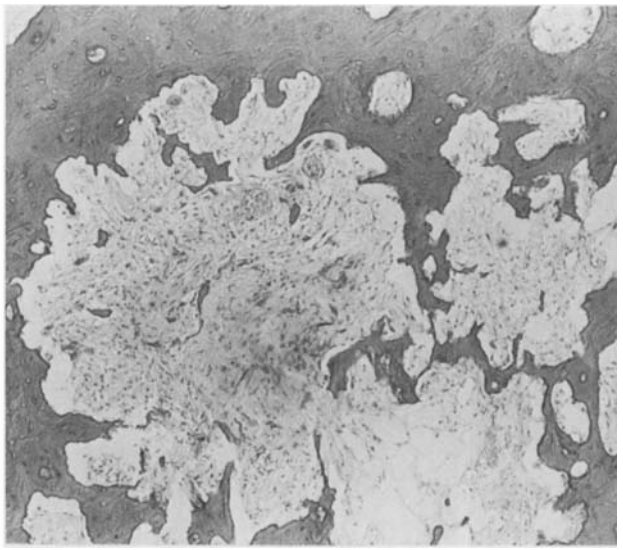


Fig. 5. Cross section, mid-diaphysis, femur. ^{90}Sr-fed dog (D60) dead at an age of 2.2 years. Large cavity complex, with rugged surfaces. Upper part osteoclastic activity. Content of the cavity, bone remnant, fat tissue, slight cellular proliferation, and large dilated vessels upper, left mid-part of the section. Mean skeletal dose 14.43 Gy. H-E ×50.

compression fracture. Pathologic fracture was noted in these cases. Reactive bone surrounding the fracture was assumed to be callous. Including the 2 tumors not evaluated radiographically, the lesions were distributed: 2-C1, 1-C2, 1-T1, 1-T2, 1-T10 and 1-L4. The lesions were all evaluated histologically as osteosarcomas.

Ten tumors failed to fit radiographically into previous described patterns. All the tumors appeared highly destructive radiographically and resembled the radiographic appearance of spontaneous primary bone tumors (22). There was no evidence of these lesions having passed through a stage of benign appearing cortical lucency before development of the more malignant appearance. The tumors were located: 2—scapula, 2—skull, 2—humerus, 1—distal femoral condyle, 1—7th coccygeal vertebrae, 1—right 8th rib, and 1—rostral mandible. Four of these were hemangiosarcomas (3 metastatic), while the others were: 1—fibroblastic osteosarcoma, 1—osteosarcoma, 2—osteoblastic osteosarcoma, and 1—eburnating osteosarcoma. One liposarcoma had typical punctate lucencies as expected in a spontaneous tumor of this type. Three of these dogs had been injected with the ^{90}Sr.

Microscopy. In this investigation, the main effort was devoted to studying different aspects of ^{90}Sr-induced lesions in bones. Most frequently involved in the induction of bone tumors were the long bones (humerus, femur and tibia), the mandible, and the ilium (Table 2).

On the microscopic level, a most conspicuous finding was the high frequency of severe lesions almost totally restricted to different levels within the compact bone of the diaphysis, whereas cancellous bone generally was much less affected. In the bone marrow cavity, there was usually a more severe cellular depletion in the central parts of the diaphysis than in the spaces between spongy bone. Very few indications of repair and cellular proliferations were obvious in the bone marrow cavity or along endosteal linings. When they were found, they were restricted to a slight, fairly paucicellular fibrosis and/or rarely, a subendosteal apposition of a small amount of newly formed bone.

On account of these observations, the main emphasis of this report is the study of the lesions occurring in compact bone, particularly with reference to the pathogenesis of osteosarcomas.

Regressive events. One of the main findings on perpendicular, or longitudinal, sections through compact diaphyseal bone was the occurrence of cavities, that varied with dose in terms of frequency, appearance, size and type. Three types of such porosities were clearly discernible: the first type mainly encompassed a large number of differently

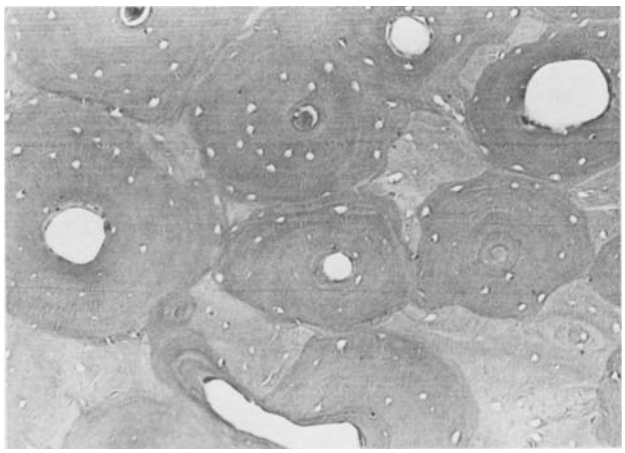


Fig. 6. Mandible, D40 level. Dog died at an age of 14.5 years. Mean skeletal dose 5.29 Gy. Devitalized osteonal bone H-E ×125.

sized, widely separated, multiple cavities, often localized within groups but without interconnection (Fig. 4). Their inner surface, fairly regular form, and microlocalization seemed to indicate that they were originating from haversian canals, whose lumina were greatly expanded. The second type, seen in other cases, or sometimes in other areas of the same section, consisted of porosities composed of large complexes of intercommunicating, strongly ramified spaces traversed by remaining bridges of previously formed bone. These cavity complexes seemed to be an older or more advanced form of those described as type one. The third type usually comprised a solitary cavity without intercommunicating bone bridges. These had irregular shapes and extremely uneven surfaces, indicating and even showing bone destruction by osteoclastic activity (Fig. 5). All types of these cavities were generally located centrally within the compact bone, but could, in advanced cases, break through the inner concentric lamellae into the bone marrow cavity. The histology of the bone surrounding the porosities varied with dose and time of exposure from normal to that of a highly devitalized bone.

In order to evaluate the relationship of time and radiation dose to the appearance of the destructive events in bone, it was necessary to analyze the normal frequency of cavities within the aging bone. This was performed at random on cross sections of the mid-diaphysis of the humerus from normal, unexposed dogs. Within a time interval between 1.2 and 17.2 years of age, there was a slight but somewhat invariable increase of small rounded holes in the compact bone representing widened haversian canals. This was, however, not clearly evident until an age of over 12 years, and became prominent around 15 years, when a fairly high number of whole haversian systems were transformed into punched-out-like cavities. The cavities were generally diffusely spread, and were usually more numerous in the central and inner parts of the bone than in the periphery. Complex confluent cavities were not seen. The inner concentric lamellae were usually diminished after 12 to 13 years of age.

In the ^{90}Sr-treated dogs, cavities did not appear earlier in the D10 dogs, but seemed to be more numerous at an age of 13 years than in the normal dogs. In the D20 level, fairly large cavities were found somewhat earlier (around 11 years of age); in the D30 level, they were already present at 7 years of age. In the two latter series, intercommunicating cavities could also be found.

In the D40 and D50 series, cavities were almost completely lacking at early ages. Instead, the haversian canals seemed narrower than normal and were often surrounded by a rim of bluish stained bone. The osteonal system was usually well marked but quite a few haversian canals were plugged. At some places the interstitial lamellae were showing signs of devitalization and osteolysis by a granular disintegration. Confluent and/or strongly irregular centrally situated cavities were found in the D50 series at 7 years and in the D40 level much later and to a lesser extent. In the D60 dogs, large cavities were found already at an age of 1.4 years (the earliest available). This analysis seems to indicate the existence of an almost sigmoid relationship between the frequency and size of the porosities on one hand and the age and dose on the other.

At sites of the bone where cavities were not predominant, the D50 dogs often showed plugged haversian canals and distorted, irregular osteons of greatly varying size. At some sites there was a striking tinctorial difference between scattered pinkish osteons and bluish devitalized areas where osteons were lacking and the bone seemed to undergo osteolysis from a diffuse granular disintegration to overt small cavities with a homogeneous pinkish content.

The irradiation effect on the osteocytes was generally difficult to evaluate because of loss or damage inflicted on these cells during the histologic procedures. A comparison with the normal material indicated that pyknotic osteocytes occasionally could

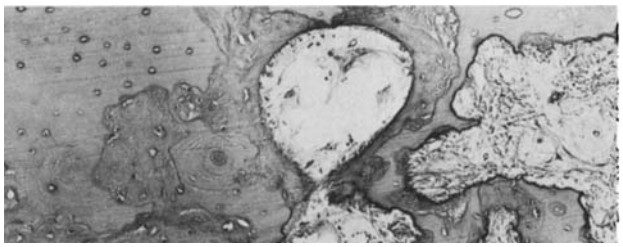


Fig. 7. Cross section of shaft of tibia, dog, level D60, average skeletal dose 14.48 Gy. Age at death 2.5 years. From corner of confluating cavity system, showing heavily disturbed bone remodelling. Filling with dark stained immature bone and paucicellular fiber formation within fatty tissue almost lacking vessels. H-E ×125.

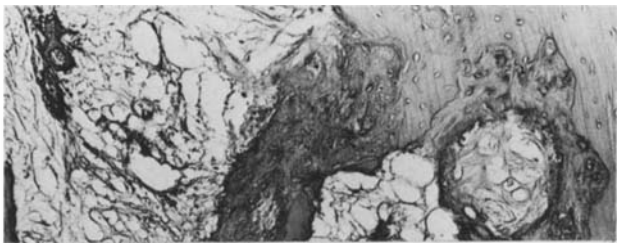


Fig. 8. Cross section, shaft of femur, dog level D60, average skeletal dose 14.48 Gy, at death aged 2.5 years. Severely impaired bone remodelling with formation of immature bone, cartilage and paucicellular fibrosis. H-E ×125.

be found in a dog aged 15.2 years in the D30 level. The damaged cells were mainly encountered within the interstitial lamellae. A more frequent occurrence of empty and widened osteocyte lacunae was found in the D40 series at an age of 10.8 years and thereafter, whereas this occurred at certain sites in the D50 and D60 levels in dogs 5 and 1.6 years old, respectively. Areas of devitalized bone found in these dose levels sometimes showed a granular disintegration of some of the osteons involved.

Progressive events. Most of the sections investigated usually revealed a complex histologic pattern in which different stages of dystrophia were intermixed with reparative dysplastic events of differing extent, appearance and age.

Almost completely reactionless, empty cavities without vascular supply were found side by side with porosities filled with fat tissue and/or bone marrow cells. On some occasions such porosities contained small to moderate amounts of fibrous tissue arranged in slender sheets. Apposition of new, almost normal looking lamellar bone was found along the walls of some cavities, but was never conspicuous (Fig. 6). More often, the preformed defects were partially filled with an immature woven bone with irregularly arranged, ill-defined lacunae. The bone at such sites differed tinctorially from the surrounding bone by a bluish color in sections (Fig. 7). There was also evidence that bone apposition had been going on earlier but had been arrested, obviously by an impaired blood supply. An indication that ischemic conditions may exist or have occurred previously was the observation, at some sites, that bone apposition had deteriorated into cartilage formation (Fig. 8). These findings seem to indicate that attempts to restore damage in haversian systems may be arrested and terminate in 'abortive regeneration'. That cavities tightly covered with active osteoblasts always seemed to have a rich supply of well filled blood vessels may indirectly emphasize the possibility of such a development (Fig. 9).

A dysplastic fibrous proliferation of an apparent variation of differentiation seems to be a predominant route of repair and filling of the large majority of the porosities. The amount produced and the histologic appearance of these dysplastic lesions could vary a great deal between cases, from area to area of the same bone, or within the same lesion. Histologically, the following types could approximately be identified:

A dense, coarse-fibered collagen-rich paucicellular fibrotic tissue, sometimes showing tendencies to form an immature bundled bone. This tissue was occasionally found to have a telangiectatic pattern by the rich occurrence of blood spaces surrounded by fibrotic 'interstices' (Fig. 10).

A coarse-fibered and, at some sites, fairly cell rich 'radiation fibromatosis', which seemed to exert aggressive potential without definite morphologically malignant characteristics (Fig. 11).

A diffuse or paramural proliferation within the porosities of fusiform and reticular cell elements successively forming condensed areas of tightly packed cells showing pleomorphism and atypical mitotic figures (fusiform, diffuse proliferation) (Fig. 12).

A fibrous-osseous repair, with a direct formation of an immature bone within a fibrous tissue without clearly identifiable osteoblasts and without malignant features (Fig. 13).

Presarcomatous lesions. The histologic appearance of the tissues filling up the bone porosities may all be indicative of a highly defective repair mechan-

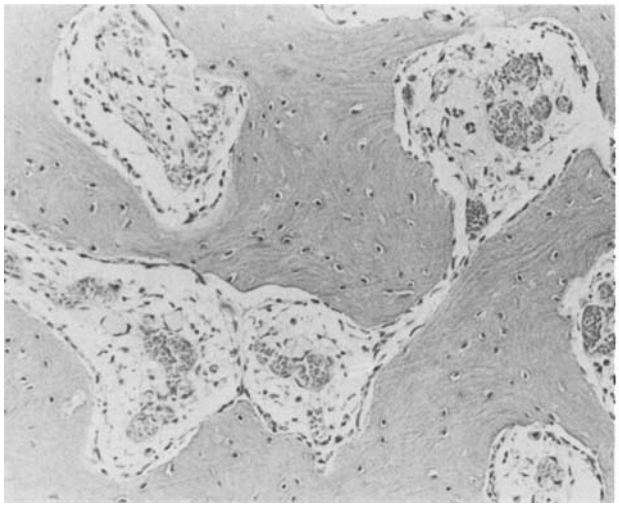


Fig. 9

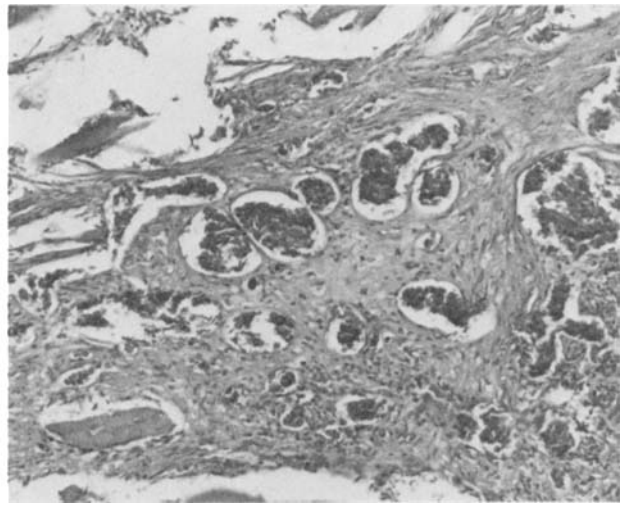


Fig. 10

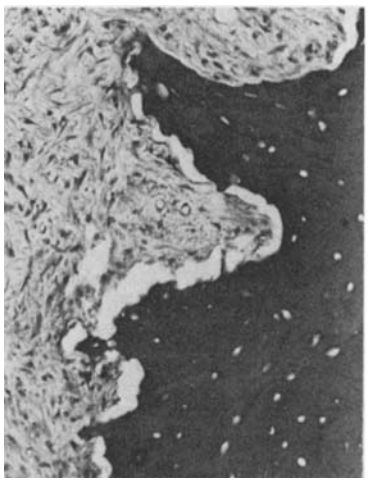


Fig. 11a

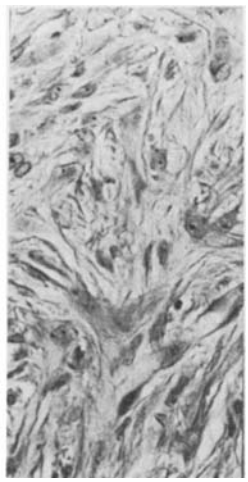


Fig. 11b

Fig. 9. Cross section, tibia, shaft. Dog aged 7.6 years at death, level D50. Average skeletal dose 13.48 Gy. Very well vascularized cavities, the walls of which are covered with abundant osteoblasts. Preformed bone well preserved. H-E ×125.

Fig. 10. Cross section, diaphysis, humerus. Dog, level D50, death at an age of 8.8 years. Average skeletal dose 13.89 Gy. Part of large cavity containing teleangiectatic coarse fibered fibrosis forming immature bone. H-E ×125.

Fig. 11. Cross section, proximal diaphysis, tibia, level D50, dog aged 7.4 years at death. Average skeletal dose 16.86 Gy. Periphery of large cavity containing a moderately cellular, fiber rich tissue attacking preformed mostly devitalized bone. The tissue has features in common with an aggressive fibromatosis. a) H-E ×125. b) ×312.

ism with potentialities that may lead to a future malignant transformation. A successive follow-up of the future development of the 'fusiform, diffuse proliferation' showed that this type of dysplasia originated within empty cavities from diffusely scattered cells. These, with time, became increasingly loaded with cells. At solitary or scattered sites within this tissue, islands of tightly packed cells might appear, some with early definite malignant characteristics. As the islands expanded they could confluate and create a fairly compact tissue with the capacity to form osteoid (Fig. 12c).

The fibrous osseous repair with early bone formation seemed histologically to represent a precancerous lesion, which with time may attain malignant characteristics.

Intracortical microosteosarcomas. This investigation clearly reveals that the intracortical tumors have a close association to the reparative events taking place within the porosities of the damaged bone by a continuous de- or dys-differentiation. Four intracortical tumors were found, 2 being of predominantly fibroblastic (Fig. 14) and 2 of osteoblastic type (Fig. 15). Three of the tumors were found in the D60 level and one in the D50 level.

Classification of overt tumors. The osteosarcomas found were subclassified in accordance with Table 3. As shown, there are no major dose-related differences with regard to the various subtypes. The most frequent subtype was the osteoblastic osteosarcoma which, in the majority of cases, was moderately boneforming, except for one case composed of extremely compact bone (eburnating osteosarcoma). Fibroblastic osteosarcomas characterized by

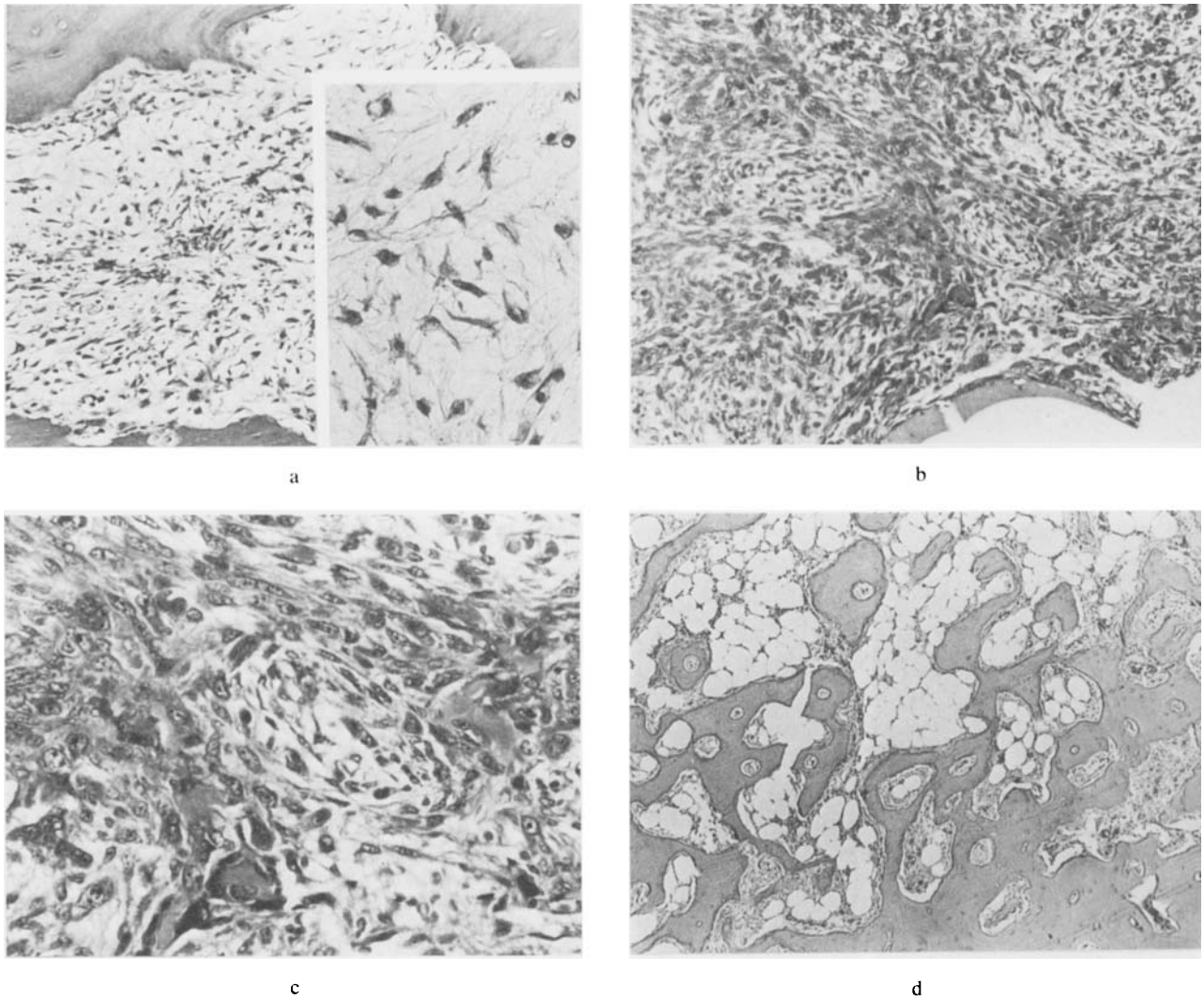


Fig. 12. a) Cross section, diaphysis humerus, level D60, dog aged 2.4 years at death. Average skeletal dose 12.55 Gy. Moderate diffuse proliferation of loosely arranged fusiform and reticular cell elements. H-E ×125. Insert ×312. b) Cross section, diaphysis, femur, dog, level D60. Dead at age of 2.2 years. Average skeletal dose 14.43 Gy. Advanced stage of process like (a), showing highly cellular tissue of tightly packed fusiform cells and areas of cellular condensation. H-E ×125. c) Magnification of (b). In center a storiform-like patterns, osteoid formation. In condensed area to the left highly pleomorphic cells. A few mitotic figures. H-E ×312. d) Cross section of diaphysis, humerus, dog level D50. Dead at age of 8.8 years. Average skeletal dose 13.89 Gy. Comprehensive paramural proliferation of slender, fusiform cells along surfaces of preformed bone trabeculae of a complex cavity containing bone marrow cells and fat tissue. H-E ×50.

an undifferentiated fibroblast-like tissue with the ability to produce bone were the second most frequent type, followed by mixed osteosarcomas (Fig. 16), chondro- and osteoclastic types. Both the hemangiosarcomas and the liposarcomas found are considered to have been induced by ^{90}Sr.

Discussion

The most conspicuous observation in the present dog material is the marked preponderance of the main pathologic lesions within the compact bone generally in the mid-diaphysis or in its close vicinity in the long bones, leaving the bone marrow cavity, cancellous bone, and the endosteal linings rather less affected. This finding is important and in close agreement with that reported by POOL et coll. (36) in their material on Ra-injected beagle dogs.

The findings in the dog on many points appear to deviate from those observed in mice (11, 24). In mice, the main lesions are instead found at several different sites along the endosteal linings or within

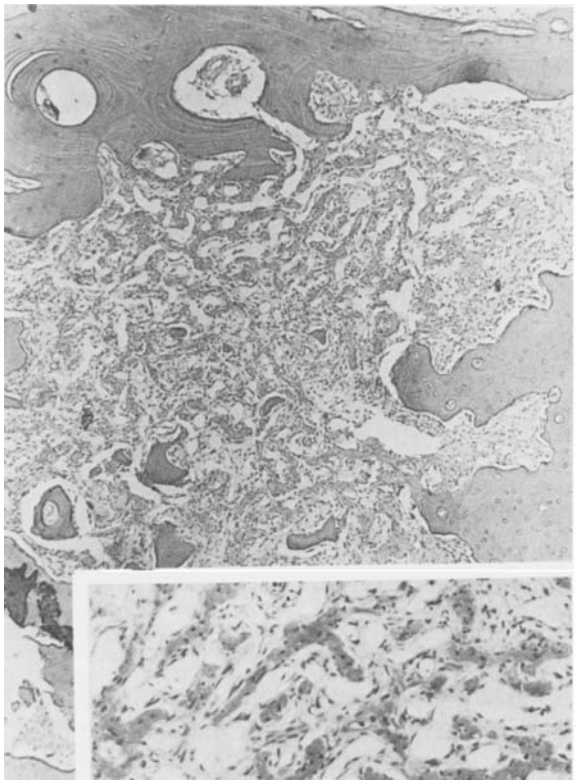


Fig. 13. Cross section, diaphysis, tibia, dog, D50 level, dead at age of 8.6 years. Average skeletal dose 12.25 Gy. Cavity with fibrous, osseous response. H-E ×50. Insert ×125.

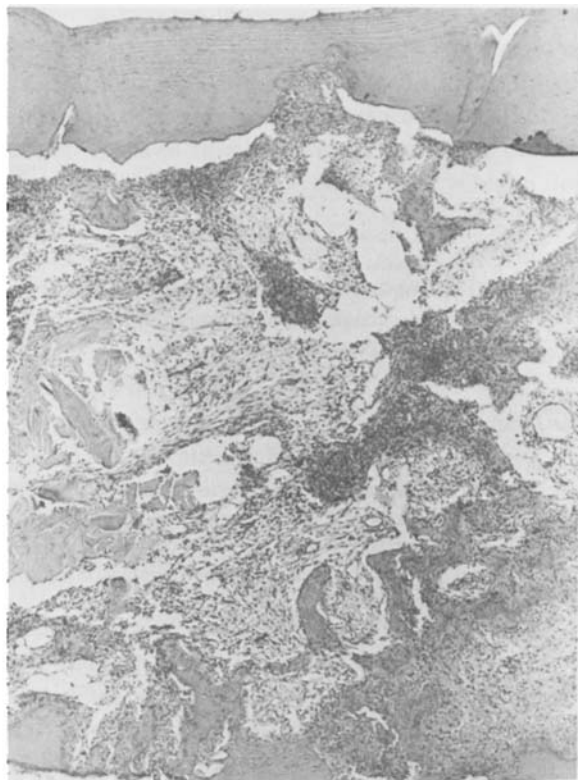


Fig. 14. Cross section diaphysis femur, dog, D50 level, dead at age of 8.4 years. Average skeletal dose 15.62 Gy. Intracortical microosteosarcoma of predominantly fibroblastic type. H-E ×50.

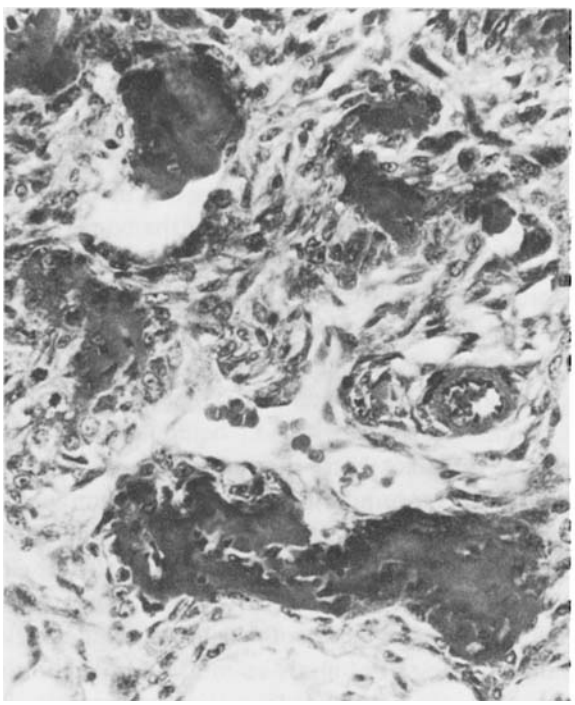


Fig. 15. Mandible, dog, level D60, dead at age of 2.2 years. Average skeletal dose 14.43 Gy. Osteoblastic micro-osteosarcoma. H-E ×312.

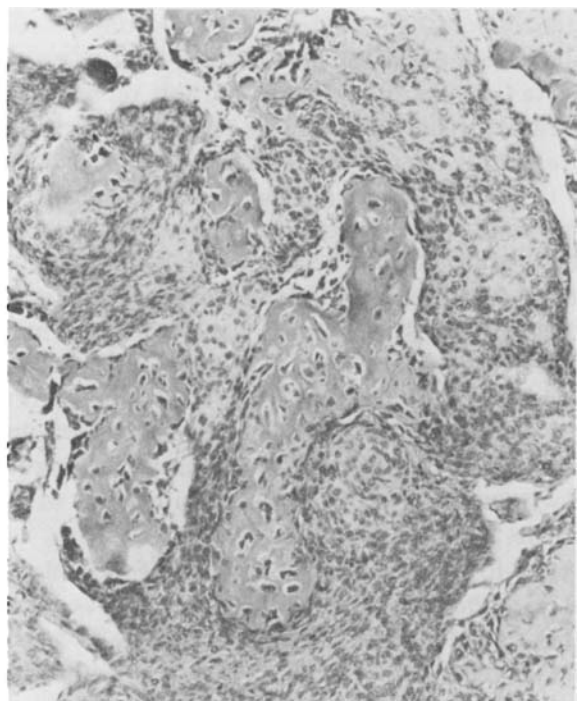


Fig. 16. Cervical vertebrae, D50 level, dog dead at age of 7.6 years. Average skeletal dose 13.48 Gy. Mixed osteosarcoma. H-E ×125.

Table 3

Classification of overt and microscopic tumors originating within bone

Tumor type	Dose level							
	D60	D50	D40	D30	D10	S40	S20	Total
Osteoblastic osteosarcomas	9	9[1]	2	–		1	–	21
Fibroblastic osteosarcomas	6[2]	5[3]	–	–	1	1	2[4]	15
Chondroblastic osteosarcomas	1	–	–	–		–	–	1
Osteoclastic osteosarcomas	–	1	–	–		–	–	1
Mixed osteosarcomas	2	3	1	–		–	–	6
Not classified osteosarcomas	1	3	1	1		1	1	8
Hemangiosarcoma	1	–	–	1		2	–	4
Liposarcoma	–	–	1	–		–	–	1

[1] Includes 1 partly teleangiectatic osteoblastic osteosarcoma.
[2] Includes 1 highly pleomorphic fibroblastic osteosarcoma.
[3] Includes 1 telangiectatic fibroblastic osteosarcoma.
[4] Includes 1 telangiectatic fibroblastic osteosarcoma.

the bone marrow cavity. There is generally a more or less obvious depletion of osteoblasts and a formation of cavities by osteoclastic activity endosteally and/or in the metaphyseal region. Occurrence of empty osteocyte-lacunae or necrotic bone is never prominent. As trabecular and cortical bone is broken down from the endosteal surfaces, it is later replaced by a fiber-rich paucicellular tissue, which gradually seems to condense into a strongly basophilic coarse fibered bone enclosing only a few osteocyte lacunae. In all likelihood, this represents a reparative process much of the same type that takes place in the dog skeleton within the compact bone, and that later, with increasing stimulus of proliferative events, may terminate as tumors at different sites.

In mice, a prerequisite for a diffuse proliferation of fusiform cells later taking place within the bone marrow seems to be a more or less complete aplasia, within which stem cells with the ability to form bone are stimulated to proliferate. Also, this lesion has close similarities to the diffuse proliferative events that are found within empty cavities in the compact bone of the dog.

Apart from the different locations of the lesions, that is, in the dog mainly in the compact bone of the diaphysis, and in mice, at different sites along the endosteum in the metaphysis or in the bone marrow cavity, the basic mechanisms of the bone destruction seem to be principally the same: bone replacement is unable to keep up with the bone destruction. This might be achieved because osteoblasts seem to be more vulnerable directly or indirectly to irradiation than are osteoclasts (7, 26). This may result from a functional defect or a numerical depression of the osteoblasts related to age and/or irradiation or an osteolytic process obviously unrelated to osteoclastic activity. The difference in location seems rather natural, taking into consideration the anatomic differences between the bone structure of the 2 species. The small size of the mouse bones makes a well developed osteonal bone rather unnecessary or at least minimally required, and therefore seems to be the main reason for the differences found.

These changes, whether in dogs or mice, are end results of a profound disturbance of the bone remodelling machinery. It may be speculated whether they are produced mainly by an indirect effect via an impairment of the vascular supply, as indicated by JEE & ARNOLD (13) for radium-treated dogs, or by a direct killing of the bone cells. The cells, as well as the vascular channels within an osteonal system or a cavity of compact bone in the dog, may certainly sustain a far more hostile radiation environment at equal ^{90}Sr concentrations than corresponding structures along the endosteal surfaces in mice, since, for a given concentration of ^{90}Sr, much more of the beta energy would be absorbed in the canine skeleton than in the skeleton of the mouse (35). Still, the endosteal osteoblast population is significantly depleted (Fig. 17) shortly after administration of ^{90}Sr at optimal or supraoptimal tumor doses (26), even

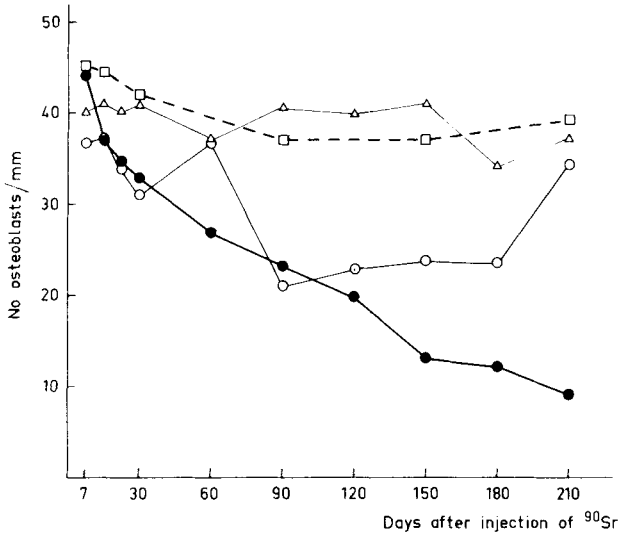


Fig. 17. Number of osteoblasts per millimeter endosteum of distal metaphysis of femur related to dose and time. Controls (□). 14.8 kBq (△). 29.6 kBq (○). 59.2 kBq (●). There was a significant difference between the 29.6 kBq and 59.2 kBq groups at 7 days ($t_{df}98=2.392$, $p<0.025$), 60 days ($t=3.560$; $p<0.025$), and 150–210 days (t varied between 4.310 and 6.471, $p<0.001$). The 14.8 kBq group differed significantly from the 29.6 kBq group at 21 days ($t=2.352$, $p<0.001$) and 180 days ($t=2.204$, $p<0.05$) after injection of ^{90}Sr (26).

though the bone marrow cavity usually is well supplied with blood, at least from disrupted sinusoids. This seems to indicate that a direct killing of osteoblasts may occur in mice and probably also in dogs.

On the other hand, in cases where the bone marrow cavity is necrotic, as is sometimes seen in the central diaphysis of mice, the bone structure of the compact bone is well preserved, probably through vessels from the periosteal side. In dogs, where the vessels are distributed for a very long distance within the canals of Havers and Volksmann, the irradiation exposure may imply a very high risk of vascular damage. Even if such lesions are scant or difficult to observe, the accidental findings of an immature cartilage within reparation sites may indicate an ischemic condition (Fig. 8). This may also be supported by the observation that in cavities, where the blood supply is richly developed, numerous osteoblasts are usually found along the bone surfaces (Fig. 9). It therefore seems plausible to anticipate that the lesions in the dog, which are produced to a higher extent than in the mouse, are caused by a combined effect of irradiation-induced damage and ischemic conditions. An indication of this may also be the greater inclination in the dog to respond with fibrosis than does the mouse.

After a certain latency period, there is in both dogs and mice a reparative response that may vary considerably, both qualitatively and quantitatively. From a qualitative point of view, four rather well defined types of response restricted to the damaged areas of the compact bone were discernible in dogs: 1) a paucicellular, coarse fibered fibrosis with the ability to form immature bone, 2) a more cell-rich, aggressive type of fibrosis, 3) a loosely arranged diffuse proliferation of fusiform, slender cells, and 4) a fibro-osseous response with a direct formation of spicules of bone.

In mice, these events had more widespread distribution, as indicated below:

A) At localized sites, as apposition of immature bone essentially at any point along, or between, trabecular bone of the metaphysis (24).

B) As multiple, endosteally located, nestlike agglomerations of large rounded cells (11).

C) Paraendosteally, as a linear condensation of loosely arranged proliferating fusiform cells usually within the midshaft of tubular bones (3).

D) Within the bone marrow cavity as islands of bone, most frequently from an area about 1 mm distant from the epiphyseal plate and without contact with the surrounding bone (24, 26).

E) Within the bone marrow cavity, preferentially in the mid-diaphysis of aplastic bone marrows as an initially diffuse, later focally scattered proliferation of fusiform and/or reticular cell elements (24, 26, 40).

F) Within cavities of compact bone, as a fibro-osseus response or a proliferation of fusiform cells (3, 11).

The 'starting points' given for malignant or potentially malignant growths have been collected from a very large amount of material from mice. In spite of that, some of the changes (mainly B and F) are seen only on fairly rare occasions. It should, however, be pointed out that the F-type lesions are seen quite frequently in mice treated with ^{241}Am (28).

The dog material is rather small by comparison, and in no way is sufficient to draw the conclusion that progressive events with malignant potentialities are restricted to porosities within the compact bones.

Apart from the dissimilarities between mouse and dog regarding the place of the main lesions induced by ^{90}Sr, many of the histologic events within the porosities of the compact bone of the dog may have a close resemblance with those of mice. The fibrous

paucicellular tissue seems to occur much more frequently and abundantly in the dog than in the mouse. In the mouse, this response is most often seen in the metaphyseal areas but seems to be more rapidly transformed into tumor bone than in the dog. The direct apposition of 'buds' of malignant bone, which occurs endosteally in mice seems to be a less frequent event on the surfaces of the walls of the cortical cavities in the dog.

On many occasions, the porosities in the compact bone of the dog are filled with a tissue showing a remarkable histologic resemblance to the diffuse proliferation of fusiform cells found within the bone marrow cavity of the mouse. This tissue has, with a high degree of certainty, acquired presarcomatous potentialities. Its development in the dog also has common features with that of the mouse, such as the initial diffuse proliferation, a later cellular condensation at solitary or multiple sites, ability to produce atypical cells, and finally, close resemblance to a low differentiated sarcoma, were it not for the ability to form tiny plaques of bone, justifying the diagnosis of fibroblastic osteosarcoma. It is pertinent to point out that intramedullary growths of this type, 'fibroblastic buds' as well as 'osteoblastic buds', are capable of autonomous growth, when microdissected and transplanted subcutaneously or intraperitoneally to syngeneic mice (23). From the transplantation sites, tumors with a close resemblance to the parent tissue develop within 3 to 4 months after transplantation. This shows that once formed, these intramedullary 'tumor buds' can follow their course of development in an environment free of ^{90}Sr (23, 25).

The nature of the fusiform and reticular cells comprising the diffusely proliferating tissue seems most likely to be low differentiated osteoprogenitor cells. Their morphologic appearance is very much like the slender osteogenic cells that do proliferate along bone surfaces in fractured bones. They may, both in dogs and mice, demonstrate an ability to form bone, particularly when approaching bone surfaces. Some insights of the origin of these cells may also be achieved by studies of the combined effects of ^{90}Sr and estrogenic hormones on the skeleton of mice (29). This hormone induces an intense formation of new bone in mice by stimulating stem cells in the bone marrow to differentiate into osteoblasts (39), proving that osteoprogenitor cells, at least in the mouse, are distributed within the bone marrow. Their osteogenic potentials will, however, be strongly evidenced mainly at sites where the cells are approached by bone surfaces growing out from the endosteum (39). When estrogen is combined with ^{90}Sr, the bone apposition is largly inhibited and instead of osteoblasts, fusiform cells do predominate, which seems to be caused by an irradiation-induced differentiation arrest.

Finally, it also seems salient to point out that diffusely proliferating cells often occupy bone cavities situated in a zone around an overt tumor. This may be interpreted as an indication that the tumor may have originated within such a type of tissue by a gradual dysdifferentiation.

The fibrous osseous or osteodysplastic response that has been found as a filling in some larger cavities in the dogs and sometimes also in mice may probably be considered, in agreement with POOL et coll. (36) and GOSSNER (9), as an endstage of a benign lesion approaching the borderline of malignancy by a gradual transformation. Histologically, it is sometimes difficult in both species to separate this lesion from an early osteoblastic microtumor. In mice, these lesions do occur late in the CBA strain after low doses of ^{90}Sr (11), whereas they may be found as spontaneous, early lesions (particularly in females), in the strain (NMRI) used by GOSSNER et coll. (10). In dogs, they are obviously radiation induced and late in appearance. In some instances in the dog, dysplastic lesions resembling a radiation fibromatosis with aggressive features but without clearly defined malignant features were observed. Responses of this type were never found in mice. The general appearance of the lesion may probably justify a close association to malignancy.

The tumors have been classified in accordance with the recommendations given by the EULEP Committee of Pathology (6). In the dog material, there are no differences of the subtypes of osteosarcomas related to dose of irradiation or sex. This material is, however, far too small to draw any conclusions in this respect. In mice, such a relationship does exist. This is, for example, evidenced by a higher frequency of osteoblastic osteosarcomas in females than in males (27) and also by an enhanced incidence of this type of tumor after injection of estrogenic hormones to males as compared with males not treated with hormones (30). Furthermore, in males treated with this hormone, osteoclastic osteosarcomas were far more frequently found than in non-treated males. Also, the radiation dose clearly exerts a shift in the tumor spectrum, whereby

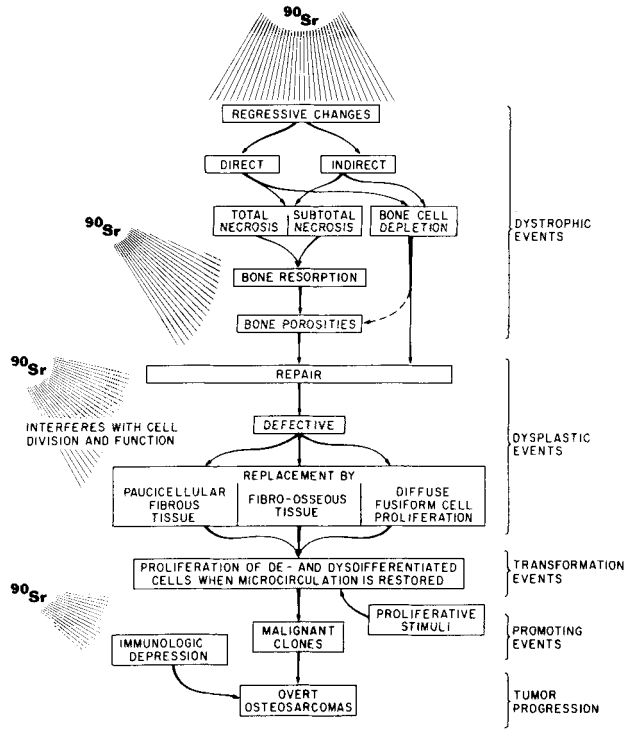


Fig. 18. Schematic representation of pathogenesis of ^{90}Sr-induced bone tumors, as studied histologically.

high doses predominantly favor the development of the osteoblastic and lower doses, mainly the fibroblastic variety of osteosarcomas (26).

It is furthermore of interest that malignant bone tumors with a vascular component (telangiectatic osteosarcomas) (21), as well as pure angiosarcomas (18, 26), were found not only in mice but also in dogs. To what extent these tumors are related to dose is not possible to evaluate in the dog material. In mice, they seem to be related both to dose (low or intermediate) of ^{90}Sr strain of mice and sex (preponderance in males) (11).

The complicated nature of the radionuclide induced injuries seems to be a result of ongoing destructive and reparative events concomitantly impaired by a superimposed irradiation. The frequency, dissemination and cellular composition of these lesions and their future development vary with the irradiation dose and factors such as age, sex, mechanical stresses, and the strain within homogeneous groups of one animal species. It is therefore not surprising to find a histologically somewhat different reaction pattern between different animal species as evidenced by number of plugged osteons, size and frequency of ischemic damage, quality and composition of repair tissue (36) or different location of tumor induction sites (24,26).

Albeit these dissimilarities, this study, as well as previous reports in mice (24, 26) and dogs and people (36) reveal that the basic events in the tumorigenesis have more unifying than separating factors. These are irrespective of animal species evidenced by a general occurrence of events, as presented in Fig. 18 and as summarized below:

Regressive events. These lesions seem to be a necessary prerequisite in all species investigated for the initiation of future tumor development. Their size, distribution, frequency, and severity also show a close relation to the future tumor rate. Supraoptimal doses of ^{90}Sr in mice (26) generally induce necrosis and devitalization at sites such as the distal parts of the femur or proximal region of the tibia, which for optimal doses are the sites most often involved in tumor development. On the other hand, there is a shift of the tumor localization pattern for supraoptimal doses to bones such as the thoracic vertebrae, which are generally less vulnerable at the optimal dose level. These also seem to be true for suboptimal doses. In these cases, the tumor induction time is considerably prolonged and the predilection site seems to be within the bone marrow of the shaft of the long bones since ^{90}Sr is more strongly retained here than in the proximal or distal parts. What is important in this connection is also the magnitude of energy absorbed. This in turn is influenced by the thickness or diameter of the various bones in the skeleton. The results of PARMLEY et coll. (35) demonstrate that energy absorption in bones of various species differs considerably. Provided that the irradiation is not directly devitalizing to the tissues, the lesions will after a certain latency time start to heal and induce:

Progressive events, which take place in an environment superimposed by irradiation and are characterized by proliferation of a defective, immature, dysplastic repair tissue. The ability of the damaged tissue to respond by cellular proliferation seems to be a very important event in cancerogenesis. Therefore, supraoptimal irradiation doses produce no or fewer tumors than somewhat lower doses, which allow the survival of cells prone to proliferate but give rise to histologically and functionally defective tissues. With time, sensitive steps of the cellular replication within these tissues are accumulating increasing doses of irradiation and therefore are ex-

posed to an enhanced risk of deterioration, de- and/or dysdifferentiation. Successively, their ability to produce a normal bone is lost and instead there is a predomination of dysplastic and hyperplastic lesions, the features of which have been described in this article. The impetus to these events may be manifold. For example, JEE & ARNOLD (13) considered the ischemic conditions in the bone to play a certain role in this respect. Furthermore, hormones may also be important both as stimulators and inhibitors, as has been shown for estrogens and glucocorticosteroids, respectively (29).

Transformation of normal cells into tumor cells. Whether the impetus to transformation is an early event lying until the conditions for its expression are optimal, or a late occasion being more closely associated with a defective repair, is not possible to evaluate in this investigation. From this material and from other works on humans, dogs, rats and mice (10, 14, 16, 20, 24, 26, 36), it is, however, possible to state that cells showing histologic criteria of malignancy in the overwhelming majority of investigations do appear first within dystrophic-dysplastic lesions.

Malignant clones. The constitution of malignant clones from transformed cells seem to provide an important link in tumorigenesis. Theoretically, however, a few transformed cells may not necessarily offer a sufficient basis for a future progression into clones and microtumors unless certain favorable conditions are at hand. Such conditions have been designated by BERENBLUM & SHUBIK (2) as promoting events and include parameters such as increased stimuli to cellular proliferation and/or an immunologic depression. In mice this seems, as previously mentioned, to be indicated by the observation that ^{90}Sr-treated female mice, for example, are more prone to develop osteosarcomas than are males and that estrogenic hormones contrary to general mesenchyme inhibitors, such as glucocorticosteroids, are highly syncancerogenic with ^{90}Sr (29). Intimately associated with the enhanced tumor yield are probably also immunologic factors. This is indicated by a three-fold prolonged skin allograft survival and an impaired primary hemagglutination response when estrogens are combined with external irradiation (41), and because ^{90}Sr induced tumors are antigenic in the mouse (31). Furthermore, BCG treatment of ^{90}Sr-injected mice has been shown to significantly decrease the tumor incidence in mice (32).

ACKNOWLEDGEMENT

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