Blood Flow and Tracer Uptake in Normal and Abnormal Canine Bone: Comparisons with Sr-85 Microspheres, Kr-81m, and Tc-99m MDP

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The bone scan, showing the 4-hr residue in bone of technetium diphosphonate, is a widely used diagnostic tool. The question arises whether it reflects changes in local blood flow to bone, or avidity, or both.

Two experimental approaches have been used in trying to answer this question. The first was to determine immediate single-passage clearance and 4-hr residue in normal and abnormal bone. If flow is the determinant of residue these should be the same since, in this event, extraction will not change. An osteotomy was produced in the dog tibia and retrograde catheterization of the anterior tibial artery allowed measured amounts of technetium-methylene-diphosphonate (Tc-99m MDP), to be infused into the nutrient artery. Similar preparation in normal bone allowed comparison of both clearance and 4-hr residue. Results have shown increased 4-hr residue 10 days after the osteotomy (normal 4.9% → 13.4%).

The second approach involved use of a gamma camera to record profiles of blood flow in normal and abnormal bone, a) by injecting Sr-85 microspheres into the nutrient artery, and b) by infusing the short-lived radioactive gas, krypton-81m, thus producing an image of regional distribution of flow. Comparison of these profiles with the distribution of Tc-99m MDP shows that a moderate increase in flow is associated with a much greater increase in Tc-99m MDP residue.

We conclude that, although blood flow is one determinant of the distribution of bone-seeking radionuclides such as Tc-99m MDP, some other determinants of clearance and residue explain their localization.

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Bone scanning with technetium-labeled diphosphonate compounds is used increasingly as an aid to medical diagnosis. Various factors have been suggested as determinants of the skeletal distribution of these compounds (*1-6*). The question arises whether the bone scan indicates the regional distribution of bone blood flow or depends on some factor that increases the avidity of bone mineral for the radionuclide, or both. Two experimental approaches have been used to investigate this problem. The first [work previously reported (*7*)] is concerned with an analysis of the extraction by canine bone of Tc-99m methylene diphosphonate (Tc-99m MDP), infused into the nutrient artery. The initial transcapillary movement and extraction of technetium-labeled phosphonates has been shown to be determined by molecular size (*8*).

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More recently an analysis of the washout curves following bolus injection of Tc-99m MDP has shown four compartments in bone with differing rate constants (*9*). The slowest of these appears to represent residual radioactivity in bone and is the component indicated by a bone scan. This residual activity, the "4-hr residue" following a bolus injection into the nutrient artery, has been compared in normal and abnormal bone. Following the induction of an osteotomy it can be shown that this residue increases from the normal (4.9 ± 1.0 s.e.m.)% to (13.4 ± 1.1)% in the bone with the osteotomy (*7*).

In the second experimental approach, profiles of blood flow were recorded in normal and abnormal bone for comparison with the Tc-99m MDP residue. The profiles were produced by: a) the injection of Sr-85-labeled microspheres into the nutrient artery of the dog's tibia and b) by using the steady-state image of the distribution of the short-lived radioactive gas, krypton-81m, as a marker of regional distribution of flow.

Theoretical considerations. *Sr-85 microspheres.* Microsphere particles have been used to estimate the fraction of cardiac output going to an organ and to measure regional flows within an organ (*10–13*). Particles greater in size than the capillary dimensions are trapped by capillary blockade during their first transit. The activity measured in any given region should therefore be proportional to its blood flow. This use of microsphere particles for measuring regional blood flow rests on the assumptions that: a) the particles are totally mixed and will travel as red blood cells do, b) the capillary blockade does not seriously alter flow to the region under study, and c) there is no portal type of circulation (double capillary network) or arteriovenous circulation.

The microspheres used in this study were procured commercially,* already labeled with Sr-85 and measuring 15 ± 5 microns in diameter.

Krypton-81m. This 13-sec, cyclotron-produced gas has been used to produce images of the distribution of regional supply in a number of organs. The principle of this technique has been applied both to the assessment of regional ventilation and to the recording of regional myocardial perfusion (*14–17*). The method rests on the establishment of a steady state, at which time the total Kr-81m counts are proportional to regional flow (F), and inversely proportional to the rate of exchange (F/M) divided by ρ, the partition coefficient between blood and bone. Regional counts are also inversely proportional to the radioactive decay constant (λ). All this can therefore be expressed as:

$$\text{Kr-81m counts} \quad \alpha \frac{F}{(F/M)/\rho + (0.693/T\frac{1}{2})},$$

since $$\lambda = \frac{\ln 2}{T\frac{1}{2}}.$$

In bone the term $(F/M)/\rho$ is very small (approximately 0.1 or less, depending on the value of ρ) in relation to the decay constant (3.2), so regional counts can therefore be considered proportional to regional flow (*16*).

Technetium-99m MDP. The bolus injection of Tc-99m MDP into the nutrient artery of a bone results in a partial extraction of the bone-seeking agent. The fraction extracted, however, is relatively low, so there is further distribution of technetium to the bone from recirculation. The resulting error, however, is relatively small and has been found in previous experiments to be approximately 5%.

MATERIALS AND METHODS

Six adult greyhounds were used for the experiments, three normal and three abnormal. The abnormal study was carried out 10 days after an osteotomy induced in the right tibia.

Arterial cannulation. The experimental setup has already been described (*7*). The dog was anesthetised and the right anterior tibial artery exposed by the transfibular approach as outlined by previous workers (*18*). A fine polyethylene catheter (0.58 mm internal diameter) was introduced into the vessel and advanced so that its tip lay at the origin of the nutrient artery. The position of the tip was checked by the injection of contrast medium (70% Urovison) angiography, which also aided location and ligation of all muscular branches arising close to the nutrient artery. This procedure has been described previously (*19*).

Induction of osteotomy. Three dogs were anesthetized and, under aseptic conditions, a vertical incision was made on the antero-medial aspect of the right tibia at the junction of the middle and distal thirds of the bone (Fig. 1). This site was chosen because it is well removed from the site of entry of the nutrient artery before it divides into upper and lower branches. The periosteum was retracted and the tibia divided with an oscillating saw. The bone was displaced and then held together with a four-holed vitallium plate, 3.8 cm long. Axial compression was applied manually at the fracture site to ensure proper internal fixation. The animals were fed 1 g tetracycline daily in their diet for 10 days, and under this regimen the operated leg became weight-bearing 3–5 days after the fracture.

Radionuclide distribution studies with the gamma camera. Images of the distribution of Tc-99m MDP, Kr-81m, and Sr-85 microspheres were recorded

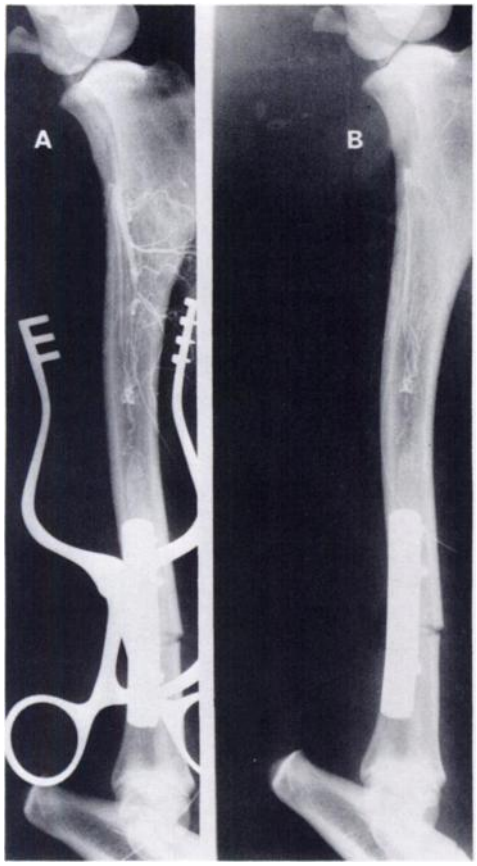

FIG. 1. (A) Bolus injection of radiographic contrast shows filling of tibial nutrient artery and muscular branches, and retrograde filling of anterior tibial artery of the osteotomy leg. (B) Trickle injection of radiographic contrast shows filling of nutrient artery only.

with a large-field gamma camera interfaced to a computer.

Technetium-99m MDP injection. Technetium-99m MDP was prepared as previously described (*20*). A known amount of activity (approximately 640 μCi) was injected into the nutrient artery and its distribution recorded 2 hr later by the gamma camera.

Krypton-81m infusion. The catheter in the anterior tibial artery with its tip supplying the nutrient artery was connected to a rubidium-krypton generator through which dextrose was pumped at a rate of approximately 5 ml per min. Under steady-state conditions the distribution of activity was recorded using the 190-keV spectral peak.

Strontium-85 microspheres. Approximately 400,000 microspheres labeled with about 20 μCi Sr-85 were injected into each nutrient artery. Images were recorded with a medium-energy collimator, using an energy setting of 510 keV.

Sectioning of bones. At the end of the scintigraphic study each dog was killed and both tibiae removed for radioassay. The bones were cut into 2.8-cm sections and each section was weighed and counted before and after removal of marrow. Counting was done at 12 cm from the face of a 15- by 10-cm NaI(Tl) detector to determine residual Tc-99m and Sr-85 activities. Simultaneous measurements were made with a dual scaler-timer unit, the signals being fed into two single-channel analyzers that were set to record the photopeaks separately. A pure Sr-85 source was counted at both energy settings to determine its background contribution into the Tc-99m window.

RESULTS

Figure 2 gives a comparison of the gamma-camera profiles of regional flow in normal and abnormal bone, as assessed by Sr-85 microspheres and Kr-81m. The results of these two techniques demonstrate similar profiles. Due to the higher energy, the Sr-85 profile is degraded by penetration of the collimator septa and this accounts for the spread of the peak. When both curves are displayed and normalized to the highest count, the distal bone represents 50% of the maximum count with Sr-85 and

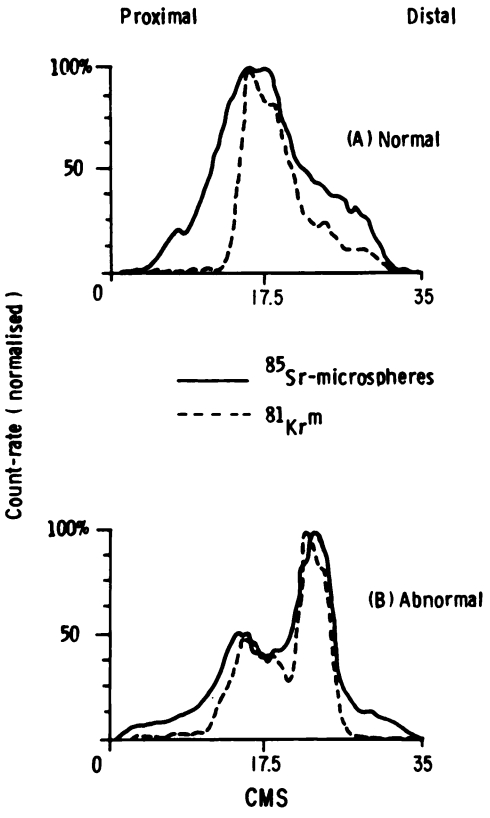

FIG. 2. Gamma-camera profiles to show distribution of Sr-85 microspheres and Kr-81m in (A) normal and (B) abnormal bone.

48% with Kr-81m (Fig. 2A). The profiles of flow in bone containing an osteotomy show disparity, again accounted for by the spread of the peak due to septal penetration with Sr-85 (Fig. 2B).

Figure 3 compares the distributions of Tc-99m MDP and strontium microspheres, plotted as activity per unit volume of bone. There are considerable differences. The profile of Sr-85 activity in normal bone (Fig. 3A) shows maximum activity at the proximal end and relatively little flow down the shaft of the bone. Technetium-99m MDP shows increased activity toward the proximal end but only a minor decrease per unit volume down the shaft. In abnormal bone, as expected, an increase of flow of approximately 100% is seen over the osteotomy (Fig. 3B, Sr-85). The increase of Tc-99m MDP over the osteotomy compared with the midshaft is 800%, and over the mean for the rest of the bone, excluding the osteotomy, the increase is approximately 400%.

Gamma images are shown in Fig. 4 for Sr-85 microspheres, Kr-81m, and Tc-99m MDP. Note the close correlation between these images and the distribution profiles shown in Figs. 2 and 3. A high concentration of activity is again seen at the proximal end of normal bone, and there is relatively little flow down the shaft of the bone as shown by Sr-85 microspheres and Kr-81m. Following an osteotomy there is an increase in both flow and residue to the distal part of the bone, the highest increase occurring at the osteotomy site itself. Figure 5 shows the contribution of bone marrow to the total Tc-99m and Sr-85 counts for normal and abnormal bone.

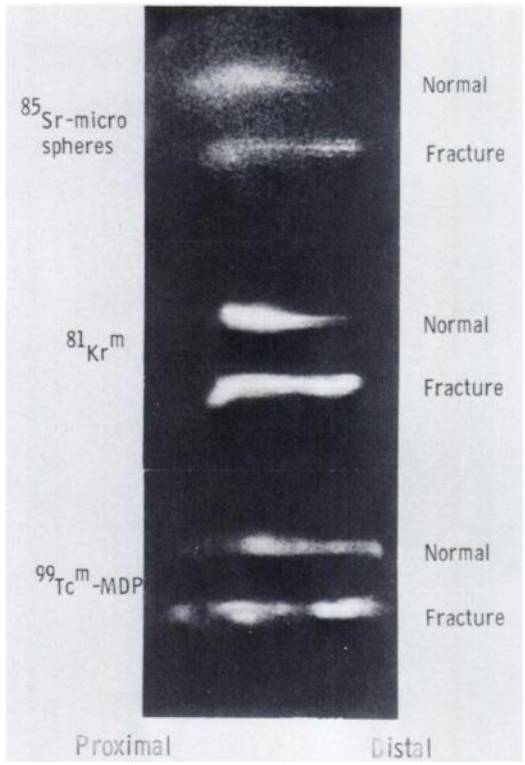

FIG. 4. Images taken with gamma camera show distribution of Sr-85 microspheres, Kr-81m, and Tc-99m MDP in normal and abnormal bone.

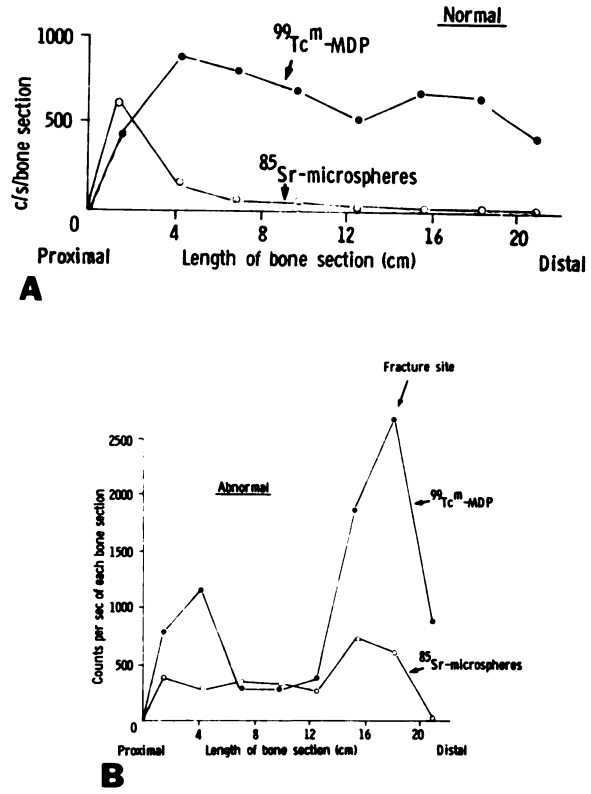

FIG. 3. Profiles of bone sections show distribution of Sr-85 microspheres and Tc-99m MDP in (A) normal and (B) abnormal bone.

DISCUSSION

The object of this investigation has been to compare the distribution of a bone-seeking radiopharmaceutical with the regional distribution of bone blood flow. In order to enhance any differences between these two, an abnormality of flow has been induced by means of an osteotomy. Previous work by Paradis and Kelly (*21*) has shown that such a lesion is associated with increased bone blood flow, maximal at 10 days. We have used two methods for recording regional flow. The method involving microspheres is well authenticated by other workers (*10–13*), but there are technical problems associated with its use in bone. To obtain total mixing, the microspheres preferably should be injected into the left atrium. In order to obtain an adequate number of such spheres in bone, however, an unphysio-

logical number would then be required.

The injection of microspheres adjacent to the organ of interest is open to criticism of poor mixing of the tracer. An attempt has been made to minimize this by injecting retrogradely into the larger vessel on the assumption that this would result in more adequate mixing. A further objection to our use of microspheres lies in the uncertainty of the blood distribution between marrow and bone. If these vessels are in series, that is, a "portal" type of circulation supplying first the bone marrow and then the bone, the microsphere method, which assumes total clearance by one capillary bed, would be invalid. We have therefore used Kr-81m to provide an independent check. The assumptions and validation of this method are given earlier in the paper. The findings that the two profiles are similar encourages one to believe that the theoretical objections to the use of microspheres outlined above are not serious here.

The contribution of bone marrow to total counts in normal bone appears low. Adjacent to the osteotomy the bone-marrow contribution increases both for flow (microspheres) and for Tc-99m MDP. Perhaps this results from the presence of granulation tissue, with early new bone formation in the medullary cavity.

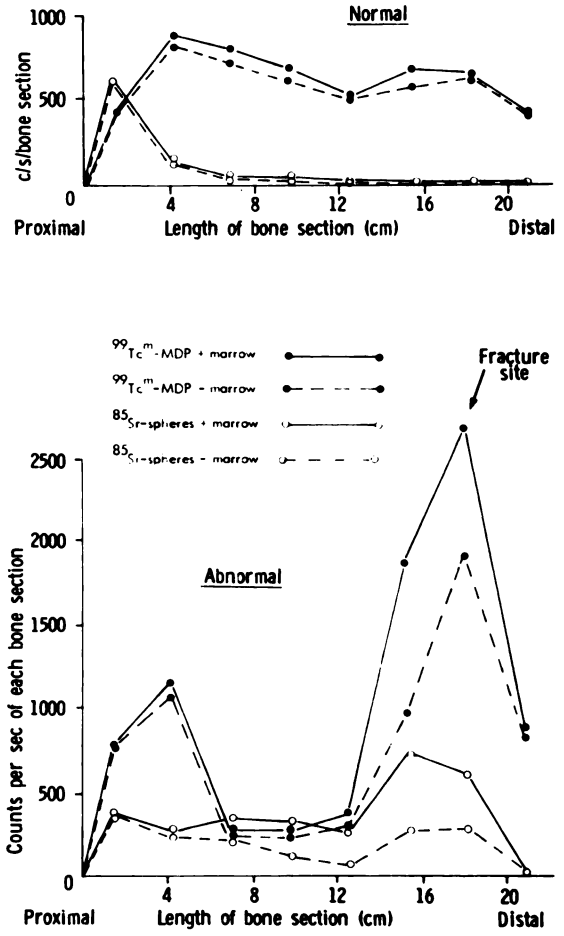

FIG. 5. Profiles of bone sections to show distribution of Sr-85 microspheres and Tc-99m MDP in normal and abnormal bone, before and after removal of marrow.

The results of comparison between blood-flow and uptake profiles show differences that are most marked in abnormal bone. It appears that a modest increase of flow is associated with a marked increase in the distribution of Tc-99m MDP. This finding correlates well with our other data concerning the increased 4-hr residue in abnormal bone (*7*). If the extraction of a bone-seeking radionuclide such as Tc-99m MDP were governed totally by blood flow, this residue should not alter, since it would be determined by the proportion of the tracer leaving the vessel, regardless of the number of vessels involved. This state of affairs appears to hold for instantaneous extraction (*8*) but not for the 4-hr residue, which is increased threefold. These two pieces of evidence therefore suggest that the distribution of Tc-99m MDP is governed by the regional distribution of flow to bone and also by some other factor related to the nature of the bone matrix.

These results differ in some respects from those obtained by Siegel et al. (*22*). Their findings of correlation between flow and Tc-99m diphosphonate increases are similar to ours but differ in the relative proportions. Thus they found a roughly linear correlation between flow and diphosphonate uptake, in contrast to our findings presented above. However, both the experimental animal (rat) and the method are markedly different, and this may explain these disparities.

Garnett et al. (*3*) have suggested that the increased uptake by abnormal bone may well be due to the combined effects of increases in both local blood flow and extraction efficiency, and that the latter might depend on factors involving changes in capillary permeability.

Galasko (*23*) has put forward the view that the major determinant of the distribution of the bone-seeking tracers is the proportion of osteoid and woven new bone. He showed by means of autoradiography that both F-18 and Tc-99m phosphate compounds localized on newly formed bone in response to experimental bone tumors, and he has also investigated the new-bone response adjacent to human neoplasms occurring in bone. He has further suggested that radionuclide uptake in bone is a two-stage process determined by the entry of the radionuclide into the extravascular space followed by bone uptake (*24*).

A similar view is taken by Charkes and coworkers (*25*). They have put forward a new model of

fluoride kinetics based on five compartments, consisting of blood, extracellular fluid space, bone extracellular space, bone, and tubular urine. They demonstrated that such a model results in only a modest increase in the bone tracer content with an increase in blood flow, but that a considerably larger increase occurs if the extraction is changed. The same authors (*26*) have investigated the effects of artifically increasing flow in dog bone and showed that in normal bone a fivefold increase in flow resulted in only a modest (70%) increase in tracer deposition.

Lastly, one might question the nature of this factor in bone causing increased extraction of tracer. It is known that the crystal in newly formed bone differs from that of mature bone in being smaller and possessing a greater surface area (*27*). We have recently performed autoradiography on specimens of a long bone exposed to Tc-99m MDP in vitro to determine whether the uptake is uniform throughout the bone (Khan et al., unpublished). The results show that, in immature animals, uptake in bone is not homogeneous but shows a marked increase in localization adjacent to the epiphyseal plate, and also in newly forming bone around the epiphyseal centers. These findings again would point to a nonuniform extraction in differing parts of bone.

We conclude, therefore, that radionuclide uptake in bone is determined by: a) regional bone blood flow, and b) a factor related to the nature of newly formed bone causing increased extraction of tracer.

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FOOTNOTE

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