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Fluid Spaces in Canine Bone and Marrow[1]

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Abstract

In order to provide a firm basis for the analysis of experiments on the kinetics of ion and solute exchange in bone, the total water space, extracellular space, and vascular (plasma plus erythrocyte) space in the tibial diaphysis in adult dogs were estimated. Tracers were injected intravenously into 40 dogs after ligation of the renal pedicles. Estimates were made of the volumes of distribution of radioactive tracers of varying characteristics (molecular weight, solubility, and charge), by taking samples of blood, plasma, and bone 3 hr after injection. The volumes of distribution (milliliters per milliliter of cortical bone) were: for tritiated water (a total water marker) 0.245 ± 0.003 (mean ± SD); for [^{14}C]sucrose (an extracellular marker) 0.043 ± 0.001; for ^{111}In-labeled transferrin (a plasma marker) 0.008 ± 0.003; and for red blood cells 0.005 ± 0.002. Interstitial fluid space was estimated by subtracting the estimated plasma space from the estimated extracellular space and it was 0.034 ml/ml of bone. Estimates of haversian canal volumes, obtained morphometrically, were 0.015 ml/ml bone and were compatible with the estimates of the vascular space.

INTRODUCTION

Fluxes of hydrophilic ions and molecules between the vascular compartment and the extravascular components of bone are rapid. A number of investigators have postulated a “membrane” in bone for rapid calcium exchange, suggesting that the cells of bone play a role in controlling ionic fluxes analogous to that played by cell membranes in other organs such as the kidney and intestines (Howard, 1956; Neuman and Neuman, 1980; Rasmussen and Bordier, 1974; Talmage, 1970).

Recent work (Cofield *et al.*, 1975; Davies *et al.*, 1976; Hughes *et al.*, 1977) has shown that diffusion is the principal mechanism for the movement of ions and molecules through aqueous passages across the endothelial layer of bone capillaries.

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A well-defined lymphatic drainage in bone has not been described. Cooper and associates (1966) have described the morphology of capillaries of cortical bone, and comparing their electron micrographs with the more general studies of Majno (1965), one would surmise that the capillaries of dog cortical bone are of the closed continuous variety. Seliger (1970), employing thorotrast, and Doty *et al.* (1976), using horseradish peroxidase (molecular weight 40,000), observed the appearance of these media in the lacunar–canalicular complex and in peripheral lacunae of the osteon. Very recent work by Norimatsu (1980) demonstrated horseradish peroxidase in periosteal vessels in rat cortical bone. Such an observation coupled with that of Owen and Triffitt (1976) and Owen *et al*. (1973) regarding an albumin measurement in bone suggests that, as in other tissues, large molecules circulate through the extravascular bone tissue in the interstitial fluid and return to the blood, perhaps by lymphatics at the periosteal surface. It may be that cortical bone contains prelymphatic vessels, which are difficult to see morphologically (Casley-Smith, 1976).

For accurate estimates of the rates of ion and solute movement in bone fluid with techniques susceptible to kinetic analysis such as the multiple indicator-dilution technique, it is necessary to have an approximation of vascular volume, extracellular fluid volume, and the cell volume in bone. It was our purpose to obtain estimates, in the diaphyseal cortex of the adult dog, of the intravascular volume, the extravascular–extracellular space, and the total water space.

MATERIALS AND METHODS

Definitions and Calculations of Bone Spaces

The volumes of distribution, milliliters per milliliter bone, of markers for various spaces in bone were calculated by using the tracer activity in plasma as a reference:

$$V = \frac{\text{activity in bone (cpm per ml bone)}}{\text{activity in plasma (cpm per ml plasma)}}. \quad (1)$$

The underlying assumptions are that there is equilibrium between bone tissue and the plasma, and that the radioactive tracer remains firmly attached to its mother substance.

For plasma space, V_p, we used both ^{99m}Tc-labeled albumin (New England Nuclear Corp., Boston, Mass., Cardiolite Serum Albumin, half-life 6.04 hr) and ^{111}In-labeled transferrin (Mediphysics, Emmeryville, Calif.; half-life 2.8 days). Albumin, with a molecular weight of 68,000, is small enough to leak out of the plasma space in bone slowly; Gregersen and Rawson (1959) estimated the loss at 8 to 10% per hour in adult dogs, and Parving and Rossing (1973) found it to be 5.2 ± 1.0% per hour in normal humans. Therefore, the transferrin was also used on the basis that its larger molecular weight (88,000) reduced the rate of loss, as was observed by Parving and Rossing (1973) for immunoglobulins. When [^{111}In]chloride is incubated with plasma, 95% is tightly bound to plasma transferrin *in vivo* and *in vitro* (Hosain *et al.*, 1969). The excess is loosely bound to albumin and α_2 and β globulins (Hartman and Hayes, 1968).

For extracellular space, V_E we used [^{14}C]sucrose (New England Nuclear Corp., half-life 5570 years, molecular weight 342), [^{14}C]inulin (New England Nuclear Corp., molecular weight ~5000), and ^{51}Cr-labeled EDTA (New England Nuclear Corp., half-life 27.8 days, molecular weight 336). Sucrose does not enter cells but does diffuse across the capillary wall readily in bone (Davies *et al.*, 1976). It does not bind in cardiac and skeletal muscle (Macchia *et al.*, 1979) and therefore appears chemically appropriate. Inulin, being much larger, would be much more strongly excluded from the interstitial fluid if the interstices of the matrix were small enough to have any effect on V_E, for sucrose and EDTA and therefore serves as a control to assess exclusion effects. ^{51}Cr-labeled EDTA is negatively charged; thus, while having the advantage of being a gamma emitter, it is subject to the possibility of binding in the matrix, as was seen for sulfate in muscle by Macchia *et al.* (1979).

In calculating the extracellular space, V_E from the inulin and sucrose spaces, allowance was made for the volume exclusion effects. In heart muscle, Schafer and Johnson (1964) and Safford and Bassingthwaighte (1977) found that inulin entered 86% of the interstitial space and sucrose 97.5%; we used these numbers since values are not available specifically for bone. The total interstitial space, for example, for sucrose, would be $(V_{suc} - V_p/(0.975)$, so that V_E the total extracellular water space, would be $V_E = V_p + (V_{suc} - V_p(0.975)$. Similarly, the estimates from inulin would be $V_E = V_p + (V_{inulin} - V_p(0.86)$. (Since ^{51}Cr-labeled EDTA appears to bind, we did not calculate V_E, from its volume of distribution.)

The interstitial space was calculated by the difference in extracellular and plasma spaces:

$$V_{\mathrm{ISF}} = V_E - V_p. \quad (2)$$

The water space, V_w, was estimated by using tritiated water, [^{3}H] H_2O (New England Nuclear Corp., half-life 12.26 years), and ^{125}I-labeled antipyrine (New England Nuclear Corp., half-life 60.2 days, molecular weight 188), which has the advantage again that it is a gamma emitter, and it appears to have a partition coefficient on bone water of about 1.0 (Kelly *et al.*, 1971).

The total cellular water plus crystalline water space can be estimated from the difference between total water space and extracellular space:

$$V(\mathrm{all\ cells}) + V(\mathrm{bone\ crystal\ water}) = V_w - V_E. \quad (3)$$

This estimate includes red blood cells as well as the cells of bone.

The erythrocyte space, V_{rbc}, was estimated by using erythrocytes tagged by the method of Dewanjee (1974); red cell hemoglobin was labeled with ^{99m}Tc. The stability of this tag was 4 to 6 hr. The activity in 1 ml of whole blood was measured, and an accounting was made for the measured hematocrit, which was corrected for an estimated 4% of trapped plasma (Owen and Power, 1953):

$$V_{\mathrm{rbc}} = \frac{\mathrm{cpm\ in\ 1\ ml\ bone}}{(\mathrm{cpm\ per\ ml\ blood})/(\mathrm{hematocrit} \times 0.96)} \quad (4)$$

From this, the volume of water in bone cells plus that in the crystalline matrix of bone can be estimated, considering that 65% of red cells is water.

$$V_w(\mathrm{bone\ cells}) + V_w(\mathrm{bone\ crystal\ water}) = V_w - V_E - 0.65\, V_{\mathrm{rbc}}. \quad (5)$$

This equation demonstrates that one cannot separate the water of bone cells from that enclosed in the structure of bone matrix (whether as crystalline water or in marrow interstices between bone crystals), and therefore bone *cell* volumes must be estimated morphometrically. V_{w} (bone cells) is considered to be provided by the estimates of lacunar volumes plus the space occupied by canaliculi. Any estimates of the water space of the bone crystal matrix [called V_{w} (bone crystal water) in Eq. (5)] therefore require estimation of V_{w} (bone cell), as considered in the Discussion.

Animal Preparation and Experimental Procedure

Forty adult mongrel dogs of random sex distribution were studied. The chronologic age was not known, but all showed closed epiphyses on X ray. With pentobarbital anesthesia, the left jugular vein and right carotid artery were cannulated in order to administer the isotopes and to collect blood samples, respectively. Direct measurements of the arterial pressure were obtained from the carotid artery. The kidneys were exposed abdominally, and all of their arteries and veins were ligated to minimize the loss of tracer (Polimeni, 1974).

Seven samples of blood of a total of 60 ml were taken during the experiment and counted for isotopic activity, In this way the plasma clearance could be plotted; 3 hr was a satisfactory equilibration time (Fig. 1).

Collection of Bone Samples

Ten minutes before sampling time, 3 hr from initiation of the experiment, the left or right tibia was exposed through a medial incision, the periosteum being left undisturbed. The wound was kept moist with isotonic saline until the time of sampling. Liquid nitrogen was then poured into the wound and over the exposed bone in order to achieve rapid freezing of the bone to be sampled. A segment of diaphyseal tubular bone was removed, with its marrow intact, by means of a Stryker saw. The time elapsing between the freezing of the bone and the tissue sampling was less than 5 sec. Subsequent experiments comparing results of sampling frozen and unfrozen bone show no essential difference in results. However, this technique simplifies handling cortical bone and marrow, especially separation of the two tissues.

The marrow was removed and weighed. Assuming the specific gravity to be 0.94 g/ml (Allen *et al.*, 1959), the volume could then be calculated. The bone was stripped of both marrow and periosteum and divided axially into two portions. One fragment was used to determine the specific gravity. The other fragment had the outer layer of bone filed away to

prevent contamination of the bone samples with periosteum. Bone, 250 to 500 mg, was then filed from the specimen, care being taken not to include any of the endosteal bone or marrow. The specific gravity, ρ, and the volume of each piece, *V*, were obtained for each sample by weight in air and water.

$$\rho = \frac{\text{weight in air}}{\text{weight in air} - \text{weight in } H_2O} \quad (6)$$

$$V = \text{weight in air} - \text{weight in } H_2O. \quad (7)$$

Equation (7) assumes a water density of 1.0 g/ml.

Isotope Counting

It was not possible to measure all of the tracer spaces simultaneously because of the number of isotopes used and the variety of techniques needed for the preparation of the samples for counting. Therefore, each calculation of the derived spaces is based on the mean of a number of determinations given in Table 1.

Gamma emitters—These were counted in a well-type multichannel analyzer (Beckman Gamma 310 system, Beckman Instruments, Inc., Fullerton, Calif.). The windows used were predetermined to measure each isotope's main energy peak or peaks for gamma emitters. The background counts were taken into account. When samples contained two gamma emitters, the observed counts were separated by means of the differential decay rates of the two isotopes or by determination of the spillover ratios of each into the other's spectral region. No special preparation of the tissue samples was required other than that already outlined.

Beta emitters—Specimens of tissue, especially those of bone, required special preparation, since it is not possible to measure beta activity in pieces of whole bone. For each tissue, quench curves were prepared. In most cases, specimens for liquid scintillation counting contained only one isotope. If they contained two isotopes, one was allowed to decay out. In one series of experiments (comparing [^{14}C]sucrose with ^{51}Cr-labeled EDTA), both a beta and a gamma emitter were present. Careful construction of quench and spillover graphs allowed the counts due to the beta emitter to be calculated with the use of simultaneous equations with two unknowns.

For the preparation of blood samples, a mixture of Soluene-350 and isopropanol, 1.5 ml, was added to 0.1 ml of blood and gently agitated; 0.5 ml of 30% hydrogen peroxide was then added, mixed, and left for 2 hr. Recovery of tracers was 99%. Fifteen milliliters of a mixture of hydrochloric acid and Instagel was then added and shaken vigorously (both Soluene-350 and Instagel are manufactured by Packard Instrument Co., Inc., Downers Grove, III.).

For plasma, a 0.2-ml sample was taken, to which 0.5 ml of water and 10 ml of Instagel were added and shaken vigorously.

The method of preparation for bone and marrow varied according to the beta emitter to be counted.

For carbon-14 the combustion technique of Schöniger (Davidson *et al*., 1970; Kelly *et al*., 1961) was used. Samples of approximately 50 mg of bone filings (Mettler H18, Mettler Instrument Corp., Princeton, N.Y.) were mixed with ground sucrose, total weight not exceeding 300 mg, and placed in a small envelope made of Spectraper membrane (Spectrum Medical Industries, Inc., Los Angeles, Calif.) and glued with Duco cement (E. I. DuPont de Nemours and Co., Inc., Wilmington, Del.). This envelope, while resting on a platinum basket, was placed in an Erlenmeyer pressure flask full of oxygen and ignited by means of a Telsa coil (Scientific Products). It was then placed in an ice bath, and 15 ml of a special liquid scintillation cocktail, containing phenylethylamine to absorb carbon dioxide, was added. A known amount of tracer was put through the same process and compared with the counts obtained by putting the tracers directly into the scintillation fluid, showing that recovery of tracers was 97%.

However, for tritium the combustion technique was found to give less than adequate recovery. In its place we used the acid digestion technique (Mahin and Lofberg, 1966). First 0.2 ml of 60% perchloric acid was added to approximately 100 mg of the bone filings, followed by 0.4 ml of 30% hydrogen peroxide. This mixture was warmed to 80° for 1 hr and then cooled. Ten milliliters of Instagel was finally added. Tests with this method showed a recovery rate of 99% or better for tritium. Standard samples in 0.2 ml were placed in Instagel and added to normal bone and prepared exactly as the experimental bone. At early times after preparing the vials there was a small percentage of counts due to the phenomenon of chemoluminescence. This was found to subside quickly, usually within 48 hr, and so all samples prepared by this method were left standing out of fluorescent lighting for at least 3 days before counting. Tritium recovery in the marrow experiments was not satisfactory—recoveries were only 40 to 60%—so that none of our data on marrow are derived from tritium.

Histologic Measurement of Osteocyte and Capillary Volume

Full cross-sectional segments from the midshaft of both left and right tibiae were taken from six dogs used in this study. The undecalcified bone was embedded in methyl methacrylate by the technique described by Jowsey (1955). Sections were then cut on a milling machine and finely ground to 40 μm on ground glass. Other sectioning techniques give thinner sections but do not give a large enough section to eliminate random distributional effects. These sections were then stained with Paragon stain (Paragon C. & C. Co., Inc., Bronx, N.Y.); a few drops of stain were placed on the section, which was then heated and cooled. The full depth of the section is not stained unless it is soaked in the stain. Usually the stain only penetrates the top 10 μm or so. The stained bone section was then mounted as usual and a coverslip was placed on it.

Photomicrographs of the stained section were taken with a Zeiss photomicroscope N°2 (Carl Zeiss, Oberhochen, West Germany) at a magnification of 250, with the use of Ektapan film and Wratten Baud G filters. The photomicrographs were enlarged 10 times, and the areas occupied by osteocytes (lacunar space) and capillaries (haversian canal) were measured by

means of a sonic Graf-pen digitizer (Scientific Accessories Corp.). Data. were placed on magnetic tape, which was fed into a PDP 1134 computer (Digital Equipment Corp., Maynard, Mass.).

Not all of the 40-μm bone section is stained, and Frost (1960) has explained how, with a similar technique, the depth of focus of the photomicroscope is extremely thin and it effectively produces a photograph of a very thin section of bone. Figure 2 shows how some osteocytes are sharply in focus and others are blurred. Only the osteocytes that were in sharp focus were measured. As a means of reducing sampling error, three areas of each tibia and six left and six right tibiae were examined. The mean for each bone, each side, and each dog was calculated for the canine tibia.

The three areas examined were just below the periosteal surface and were equidistant from each other. These sites were chosen so that they would correlate with the sites used for preparing midshaft cortical bone filings.

Frost (1960) has shown that if random distributional effects are eliminated, areas measured by this technique can be expressed in terms of volume.

Water of Desiccation

Estimates of water content of cortical bone were obtained by desiccation at 50° for 24 hr and weighing; this drives off a fraction of the water. The further weight loss by drying at 100° for another 24 hr was measured (Robinson, 1975).

$$\text{Water content (ml/ml bone)} = (\rho_B/\rho_w)\cdot\left(\frac{\text{weight before drying} - \text{weight after drying}}{\text{weight before drying}}\right), \quad (8)$$

where ρ_B for bone was determined on each sample and $\rho_w = 1.00$. Water content of marrow was previously determined in this laboratory to be 0.19 g/g (Kelly, 1973).

RESULTS

In the experiments, we found that the average increase in hematocrit (percentage) was 3.6 ± 2.9 (SD) over the 3 hr. Recordings of the blood pressure ensured that the cardiovascular status of the animals was stable over the same period. Any animals showing signs of shock were discarded.

Table 1 gives estimates of the space occupied by the tracers, that is, their volumes of distribution, V_D (milliliters per milliliter bone), at 3 hr in tibial cortical bone.

Total Water Space

In the same 4 dogs in which the tritiated water space was measured, water of desiccation at 50 and 100° was 0.210 ± 0.007 and 0.280 ± 0.005 ml/ml, respectively. This compares well with the water of desiccation for the whole group of 31 dogs, which was 0.220 ± 0.006 at 50° and 0.280 ± 0.005 mm/ml at 100°. The water space estimated by tritium with equilibration at 3 hr was 0.245 ± 0.003 ml/ml. Iodoantipyrine gave estimates of 0.216 ±

0.028 ml/ml at 3 hr equilibration; however, in 6 dogs this equilibration time was extended to 4 hr and gave values of 0.245 ± 0.037 ml/ml.

Vascular Space within Bone

Since both red cell space, V_{rbc}, and plasma space, V_p, have been approximated, the vascular space, V_{bl}, can be estimated, since

$$V_{bl}=V_p+V_{rbc}. \quad (9)$$

Taking the space defined by ^{111}In-labeled transferrin as a valid measure of plasma space, the vascular space is 0.013 ml/ml.

Interstitial Fluid Space (ISF)

The [^{14}C]sucrose space is assumed to be near to the extracellular space. With V_E = 0.043 ml/ml and V_p = 0.008 (^{111}In-labeled transferrin), this gives $V_{ISF} = V_E - V_p = 0.043 - 0.008 = 0.035$ ml/ml. Taking the average values in Table 1 for V_E (sucrose space and inulin space) and for V_p (albumin space and transferrin space), this gives $V_{ISF} = 0.043 - 0.009 = 0.034$ ml/ml.

Water Space of Bone Cells

The water of bone cells cannot be estimated by the difference between the total water space and the extracellular space because an unknown quantity of water is also held in the bone matrix. For this reason we must rely on the morphometric data for estimates of cell water.

Morphometric Data

The average volume of lacunar space in cortical bone was 1.50 ± 0.44% (SD), and for haversian canal space it was 1.47 ± 0.58% (SD). The haversian canal spaces are thus sufficient to enclose the vascular space, estimated above to be 0.013 ml/ml bone.

Marrow Spaces

Specimens of marrow were investigated in a manner similar to that used for bone. Table 2 summarizes the values.

DISCUSSION

This study presents information about physiologic compartments in bone tissue estimated by radioactive tracers of various sizes and characteristics. The anatomic validation that we offer is measurement of lacunar and haversian canal volumes by morphometry. Cell volume can be estimated indirectly by correcting lacunar volume according to Frost's (1960) estimates for canalicular volume. The accuracy of these estimates of vascular and cell volume limits attempts at validation of the physiologic compartments by morphologic studies.

The main vascular channels within bone are in the haversian canals. These passages also contain nerves and interstitial fluid, so that the vascular space should be less than the haversian canal volume but of the same order. The morphometric measurement of the canal

volume was 1.50% and the vascular space as measured by tracers was 1.3%—which are consistent with one another.

The cells of bone are found in the lacunae and canaliculi, and so the cell space should be of the same order as the total of these spaces. Our morphometric data show lacunar volume to be 1.47% by volume of the cortical bone specimen we examined. Frost (1960) observed a value for lacunae in human cortical bone of 0.80% and for canaliculi of 1.48%. If canine bone has the same ratio of lacunae to canaliculi, then canaliculi volume in dog bone might be 2.72%. This would yield a total volume for lacunae and canaliculi of 4.2%. Furthermore, a number of investigations (Baud, 1968; Baud and Morgenthaler, 1963; Vose, 1963) have described microcanaliculi of about 50 nm in diameter which radiate from canaliculi. They have suggested that this would theoretically increase canalicular volume by 100%. This would increase cell volume to about 7% in the dog; $1.47 + (2.72 \times 2) = 6.9\%$. If we use this value, one would calculate from Eq. (5) that the water associated with the crystalline bone matrix was $0.245 – 0.043 – 0.032 – 0.069 = 0.09$ ml/ml bone, although it should be understood that this is an estimate whose accuracy is low because of the multiple accumulated errors.

Our choice in these experiments of an equilibration time of 3 hr to measure the plasma space could lead to overestimates. The tracers ^{99m}Tc-labeled albumin and ^{111}In-labeled transferrin could leak out of the capillary, and this would lead to erroneously high values for the plasma space. Leakage of albumin does in fact occur in rabbit bone (Owen and Triffitt, 1976; Owen *et al*., 1973). Subsequent studies in this laboratory indicate that a smaller plasma space is obtained with shorter equilibration times (Day and Kelly, 1979). We used ^{99m}Tc-labeled albumin and an equilibration time of 30 min. In this study, Vp for ^{99m}Tc-labeled albumin was (mean ± SD) 0.004 ± 0.0006 (N = 13). Our concern is that with long equilibration times, labeled albumin may overestimate plasma space and with shorter times underestimate plasma space. The larger molecular transferrin may be the more accurate tracer for estimates of plasma space.

In vitro studies of bone were interpreted by Neuman and Neuman (1980) to suggest that inulin and sucrose were not reliable as markers for extracellular space. Part of their concern arises from the large proportion of water in bone which remains outside our estimated cell volume of 7% and the vascular space of bone. It is possible that water is held in pores in the crystal of bone as proposed by Holmes and associates (1964). They have proposed pores that are coarse (100–300 Å) and a fine pore structure of <10 Å. The large difference between the summated values for cellular space and interstitial fluid space compared with the total water space may merely reflect exclusion of sucrose and inulin from the finer pore structure of bone into which tritiated water can pass.

The difference, 0.033 ml/ml, between the ^{51}Cr-labeled EDTA space and the [^{14}C]sucrose space represents a space available to ^{51}Cr-labeled EDTA but not to sucrose. It is possible that ^{51}Cr-labeled EDTA, being negatively charged, attaches to binding sites in the matrix and therefore concentrates in bone. A less plausible suggestion is that [^{14}C]sucrose fills merely the perivascular space and that ^{51}Cr-labeled EDTA enters a separate bone tissue fluid space in addition. This, however, would mean that the postulated membrane acts to

prevent the passage of sucrose but not ^{51}Cr-labeled EDTA—an unlikely event. In view of our findings, it seems likely that the use of ^{51}Cr-labeled EDTA as an extracellular fluid space marker should be viewed with suspicion when used in relation to bone (Wootton *et al*., 1976). A better one may be ^{58}Co-labeled EDTA (Co^{3+}, cobaltic form), which has the advantage that the cobalt is much more tightly bound to EDTA than is Cr^{2-}, and in the heart it has the same volume of distribution as sucrose (Bridge *et al*., 1982).

The bone water measured isotopically by tritium was 0.245 ml/ml, and in our opinion it is the most representative value of the total bone water. The value of ^{125}I-labeled antipyrine, at 4 hr equilibration, supports this value.

The 50° desiccation for 24 hr was smaller and is presumably an underestimate, as not all the water will have been driven off. The value obtained by desiccation at 100° was higher, possibly because a nonmineral organic component might be lost as well as the water and which, if so, would lead to overestimation.

The values for marrow are much more difficult to interpret. Total water space estimated by ^{125}I-labeled antipyrine was 0.14 ml/ml at 3 hr. Previous work in this laboratory for marrow water content at desiccation temperatures of 50° gave 0.19 g/g marrow (Kelly, 1973), a figure similar to that of Dietz (1949) for dog marrow, 20 Vol%. A critical review of Table 2 reveals the relatively large standard deviations that indicate the variability in the composition of marrow.

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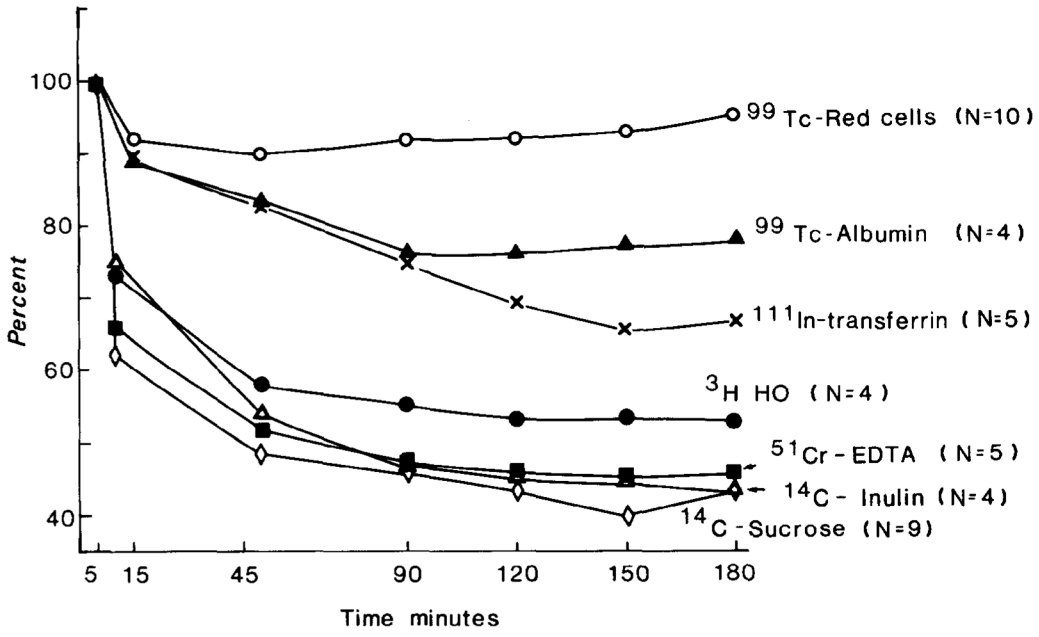

Fig. 1.

Percentage of tracer remaining in blood. Five-minute value is taken as 100%. Each point is the mean of a group of dogs for that tracer. Technetium is corrected for decay.

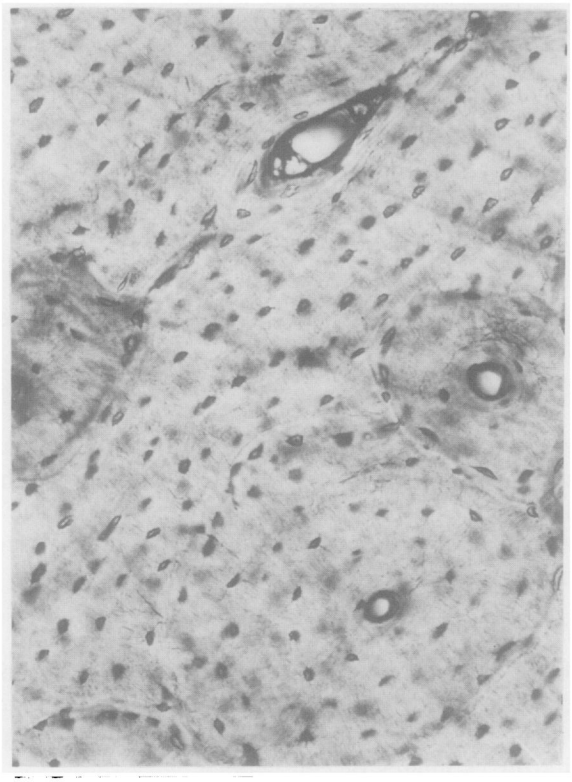

Fig. 2.
Cross section of cortical bone from tibia (Paragon; × 250). Illustration is of lacunae and their haversian canals.

TABLE 1

Volumes of Distribution (V_D) in Tibial Cortical Bone Determined in 40 Dogs at 3 hr

Space	Tracer	N^a	Mean V_D (ml/ml)	± SD
V_p	^{99m}Tc-labeled albumin	4	0.010	± 0.003
V_p	^{111}In-labeled transferrin	5	0.008	± 0.003
V_{rbc}	^{99m}Tc-labeled red blood cells	10	0.005	± 0.002
V_E	[^{14}C]Sucrose[b]	9	0.043	± 0.001
V_E	[^{14}C]Inulin[b]	4	0.042	± 0.007
V_{EDTA}	^{51}Cr-labeled EDTA	7	0.076	± 0.035
V_w	Tritiated water [^{3}H]	4	0.245	± 0.003
V_w	^{125}I-labeled antipyrine	6	0.216	± 0.028

[a] Individual observations. In some instances one dog had more than one tracer; therefore, *N* is greater than 40.

[b] Corrected for observation that inulin can enter 86% and sucrose 97.5% of the extracellular space (Safford and Bassingthwaighte, 1977; Schafer and Johnson, 1964).

TABLE 2

Volumes of Distribution (V_D) in Tibial Marrow Determined in 31 Dogs at 3 hr

Space	Tracer	*N*	Mean V_D (ml/ml)	±SD
V_p	^{99m}Tc-labeled albumin	4	0.069	±0.045
V_p	^{111}In-labeled transferrin	5	0.035	±0.012
V_{rbc}	^{99m}Tc-labeled red blood cells	10	0.024	±0.014
V_E	[^{14}C]Sucrose	9	0.091	±0.047
V_E	[^{14}C]Inulin	4	0.148	±0.114
V_{EDTA}	^{51}Cr-labeled EDTA	7	0.127	±0.108
V_w	^{125}I-labeled antipyrine	6	0.139	±0.028