Published in final edited form as:

J Appl Physiol. 1971 July ; 31(1): 38–47.

Blood flow in canine tibial diaphysis estimated by iodoantipyrine-¹²⁵I washout

Patrick J. Kelly, Tada Yipintsoi, and James B. Bassingthwaighe

Mayo Clinic and Mayo Foundation, and Mayo Graduate School of Medicine (University of Minnesota), Rochester, Minnesota 55901

Abstract

Tibial bone blood flow was estimated in dogs by an indicator washout method using ¹²⁵I-labeled 4-iodoantipyrine (I-Ap). The tibial cortical bone/blood partition coefficient, λ , of I-Ap was (mean $\pm$ SD) 0.150 ± 0.027 for 17 dogs. Tibial cortical bone blood flow (mean $\pm$ SD) was 0.0152 ± 0.003 ml/ml bone per min by the residue function technique of Zierler applied to 32 washout curves. Exponential analysis by the successive subtraction method gave the same mean value and, if the flow were considered to traverse two regions in parallel, suggested that 22% (SD, ± 7.4) of the indicator traversed a high-flow region (mean flow, 0.265 ml/ml bone per min; SD, ± 0.194) and the remainder had a mean flow of 0.0118 ml/ml bone per min (SD, ± 0.003).

Keywords

cortical bone blood flow; 4-iodoantipyrine; partition coefficient of I-Ap for bone; washout curves; residue function

Techniques to estimate bone blood flow are limited in scope and applicability in animals; to date, none has much hope of application in man. The common methods used are isotopic —⁴⁵Ca (11), ⁸⁵Sr (6,24), ⁴²K (13,14), ⁵¹Cr-labeled erythrocytes (4,25), and ¹⁸F (23)—by embolization of the arterioles of bone (5,14), attempts at quantitation of venous outflow (7), and plethysmography (10). Considering bone specific gravity to be 2 g/ml, the flows reported range from 0.01 (10) to 0.58 ml/ml bone per min (4).

The tracer or particle deposition techniques preclude repeated study in the same organ because the tissue must be removed for activity measurements. These methods are clearly valid only if extraction is 100% and if the delivery to all the tissues has been shown to be proportional to flow. Soluble tracer deposition techniques are questionable because their extraction is dependent not only on regional flow and permeation rate in the tissue but also on the hope that, when the circulation is stopped, the indicator is trapped in all regions in proportion to flow. In view of the tremendous ranges in transit times in various organs, the calculated flow probably gives the correct value only by chance and only in certain organs. Arteriovenous concentration differences and application of the Kety-Schmidt equation is theoretically valid, but it is difficult to obtain samples from osseous veins.

Washout after deposition of gamma-emitting, highly diffusible indicators that are flow-limited in their blood-tissue exchange is a useful technique because the quantity of tracer can be measured by external detection, no venous samples are required, and the analysis is simple using the height/area formula of Zierler (27) or its modification (1). Radiolabeled

T. Yipintsoi is a National Institutes of Health Special Fellow in Physiology.

J. B. Bassingthwaighe is the recipient of a Career Development Award from the National Institutes of Health.

iodoantipyrine (I-Ap) fulfills these requirements in the heart (1,17) and would be useful for bone if the bone-blood partition coefficient were known and if it could be shown to be flow-limited. If it is flow-limited, then intra-arterial introduction achieves complete entry into the tissue, which is essential for use in bone where depots cannot be made by needle injection. The purpose of this study was to measure blood flow in tibial cortex by washout techniques utilizing I-Ap as the indicator.

Methods

Three groups of experiments were performed. The first group of experiments was done to determine the partition coefficient of I-Ap between blood and bone. The second concerned the estimation of tibial blood flow by I-Ap washout; the tibial nutrient artery was used for injection of tracer. Because cannulation of the nutrient artery may disturb blood flow in bone, a third group of experiments was done to ascertain the effect of cannulation on bone clearance of ^{85}Sr . Also, in some experiments the tracer was injected via a fine needle carefully inserted into the nutrient artery, obviating the need to tie in a cannula. Subsidiary experiments were done to ascertain the effects of recirculation on the isotope washout curves, to determine the contribution of isotope contained in bone marrow to the externally recorded curves, and to determine the fraction of the isotope in marrow absorbed by the overlying bone. Lastly, experiments were performed in which the tibial nutrient artery was perfused with a known amount of the dog's own blood via a cannula and then cortical bone blood flow was estimated by washout of I-Ap after a slug injection of I-Ap was made rapidly, via a small needle, into the cannula just prior to its entrance into the nutrient artery.

All experiments were on adult mongrel dogs weighing 14–24 kg. Maturity of each dog was ensured by the absence of the epiphyseal growth plate on roentgenograms of the tibia.

Equations for Estimating Bone Blood Flow

Residue function—I-Ap washout curves were analyzed by using a modification (1) of the height/area method of Zierler (27) (see Fig. 1). The residue function, $H^*(t)$, is the fraction of the injectate remaining in the bone and therefore can be considered to be $C^*(t)/C^*(0)$, in which $C^*(t)$ is the count rate at time t , measured by an external detector placed over the bone, and $C^*(0)$ is the peak activity count rate. The peak, $C^*(0)$ at $t = 0$, occurs within a few seconds of the injection at t_0 , which is different from $t = 0$. The modified formula gives the blood flow, f (ml/g bone per min):

$$f = \frac{\lambda}{\rho} \cdot \frac{C^*(0) - C^*(T)}{\int_{t_0}^T C^*(t) dt} = \frac{\lambda}{\rho} \cdot \frac{(1 - H^*(T))}{\int_{t_0}^T H^*(t) dt} \quad (1)$$

in which T is the time of the ending of the recording, usually 15 minutes, ρ is the bone specific gravity (g/ml), and λ is the partition coefficient (the average bone/blood solubility ratio) for I-Ap in tibial cortical bone. Flow also can be expressed as f' (ml/ml bone per min), which equals $\rho \cdot f$. In these studies, flow will be expressed as f' . The integral in the denominator is the shaded area in Fig. 1 in which the vertical arrow gives the value $C^*(0) - C^*(T)$.

Two-exponential analysis—The recorded curve, $C^*(t)$, was fitted with a two-exponential equation (eq A-9 in appendix) by using the successive subtraction technique (8). A straight line was fitted visually to the tail of the recorded curve on semilog paper and extrapolated back to zero time. This line was subtracted from the original curve, the differences were plotted on semilog paper, and another best straight line was drawn through

these new points and extrapolated back to zero time. Whether or not the flow is actually through two regions in parallel, the average blood flow is given by:

$$\frac{f'}{\rho} = f = w_1 f_1 + w_2 f_2 \quad (2)$$

in which the two “regional flows,” f_2 and f_1 , are calculated from the slopes of the lines as indicated in equations A-1 and A-10 (appendix) and are, therefore, dependent on assuming the correct partition coefficients and specific gravities for the two regions; in this study they were assumed to be the same. w_2 and w_1 are the relative masses of the two regions, ($w_1 + w_2 = 1.0$). The flow estimates obtained by equations 1 and 2 theoretically should be identical.

Preliminary Experiments

Partition coefficient (λ)—The partition coefficient for I-Ap was determined when venous I-Ap isotope activity was changing and also at indicator steady state (activity of blood entering the tibia equaled activity of venous blood leaving the tibia). For these experiments, 17 adult dogs were anesthetized and heparinized, either the right or the left tibial nutrient artery and vein were cannulated (15), and an isotope detector (thallium-activated sodium iodide crystal) was placed externally over the anterolateral mid-diaphysis of the tibia as described below for determination of I-Ap washout curves. In 10 experiments, 30 μC of I-Ap dissolved in 30 ml of the dog's own blood were infused (infusion-withdrawal pump, Harvard Apparatus, Millis, Mass.) at a constant rate of 2.2 ml/min for 8–19 min, until external counting showed a plateau of count rates (Fig. 2). In seven other experiments, 50 μC of I-Ap dissolved in 75 ml of the dog's own blood were infused at a constant rate of 1.5 ml/min for 10–46 min (Fig. 3).

In each of the 17 experiments, at the end of perfusion, two 50- μl samples of nutrient vein blood were collected, the dog was killed, and three cross sections of tibial diaphysis were removed. In the first 10 experiments, radioactivity in venous blood was less than that of the infusate but, in five of the last seven there was no arteriovenous difference. The marrow was removed from each tibia, and the cortical bone specimens were cut into four smaller pieces and weighed. By serial elution of the bone pieces in 2-ml aliquots of water, nearly all ($96.4 \pm 1.4\%$) of the I-Ap could be removed in 4 hr. Each small specimen and the solution containing the eluted I-Ap were counted in a well counter (Auto Well II, Picker Nuclear, White Plains, N.Y.). The 50- μl samples of nutrient vein blood and arterial perfusate were diluted to 1.0 ml and counted in the same counter. Calculated as isotopic activity per milliliter of cortical bone divided by the activity per milliliter of venous blood, the mean partition coefficient, λ , was 0.150 ($\text{SD}, \pm 0.027$) (Table 1).

Specific gravity (ρ)—In the first 10 experiments the pieces of cortical bone that had been counted for isotopic activity were weighed (wet weight) and then dried to constant weight at 100 C for 24 hr. The volume of these pieces of bone was determined by waxing the bone lightly and measuring the volume of water it displaced. Specific gravity, ρ , was wet weight of these pieces divided by their volume. In the seven other experiments, weight loss was obtained after drying at 50 C for 24 hr and again after drying at 100 C for 24 hr. In these seven experiments the specific gravity was estimated from the weights in air and water of three sections of bone directly adjacent to those in which isotopic activity was determined ($\rho = \text{weight in air divided by difference between weights in air and water, assuming specific gravity of water to be 1.0}$). The mean specific gravity was 1.94 g/ml ($\text{SD}, \pm 0.1$) (Table 1).

Bone water—Bone water was taken to be the difference between wet and dry weights divided by the volume of the bone specimen. The mean apparent water content was 0.149 g/ml bone ($_{SD}, \pm 0.015$) after drying at 50 C and 0.256 ($_{SD}, \pm 0.070$) g/ml bone after drying at 100 C (Table 1).

Estimation of Bone Blood Flow From I-Ap Washout Curves

In 10 anesthetized, heparinized dogs, the tibial nutrient artery was isolated and cannulated as previously described (15). A sodium iodide (thallium-activated) crystal, $\frac{3}{4}$ inch in diameter and thickness and shielded with brass and lead $\frac{3}{8}$ inch thick, was placed over the anterolateral middiaphyseal surface of the tibia. This guards against the possibility that pooling of the indicator within the nutrient artery might interfere with the external counting because the detector is 4–6 cm away from where the nutrient artery enters the tibia. The pulse-height window settings were selected to detect the gamma energy between 0.0175 and 0.050 Mev.

Fifteen microcuries of I-Ap dissolved in 1 ml of isotonic saline were injected rapidly (3–5 sec) into the tibial nutrient artery. In five washout curves determined in four dogs, the isotope injection was followed by rapid injection of 1 ml of saline. These washout curves differed from the others only in that the peaks of the curves were less sharp. Calculations of flow gave values that were essentially the same as those from the subsequent 32 curves to be described in detail.

On a strip-chart recorder, isotope emission curves $C^*(t)$ were recorded from an analog rate meter (Victoreen Instrument Division, Cleveland, Ohio). One to six washout curves were recorded in each experiment and were normalized by dividing by $C^*(0)$, the peak rate; blood flow was estimated by equations 1 and 2.

The influence of the duration of the recording of $C^*(t)$ on estimates of flow was tested on nine curves (from six additional dogs) which were recorded for 30 min or more. Flow estimates were made by equation 1 using ending times, T , of 15 and 30 min to test the sensitivity of the flow estimates to the time chosen for the data collection.

Subsidiary Experiments

Use of needle injections into nutrient artery—In five additional dogs, a fine needle was inserted into the exposed nutrient artery and I-Ap was injected as described above. This method was used in order to disturb the normal nutrient artery flow as little as possible. To show that no major disturbance had occurred after completion of the washout determinations, the wound was reopened and, under direct vision, a dilute solution of methylene blue in saline was injected. In each case the artery was observed to be pulsating. The dye washed into the bone with the flowing blood; no clotting or leakage was observed near the point of insertion of the needle.

Effect of cannulation of tibial nutrient artery on deposition of ^{85}Sr —In five dogs the right tibial nutrient artery was cannulated and injected with saline without I-Ap. Ten microcuries of $^{85}\text{SrCl}$ in isotonic saline were injected rapidly through a catheter in the jugular vein. Ten 1-ml samples of blood were drawn at 1-min intervals via a catheter in the right carotid artery so that ^{85}Sr clearance could be calculated. The dog was then killed and both tibias were removed rapidly, cleaned, and counted for ^{85}Sr in a small whole-animal counting chamber. Arterial blood samples were counted in an automatic assembly (Nuclear-Chicago, Des Plaines, Ill.). For comparison, there were available from previous studies (21) 25 separate determinations of tibial ^{85}Sr clearance in adult dogs.

Recirculation—Return of washed out tracer to the observation point would decrease the apparent rate of escape and cause underestimation of flow. In three washout experiments a second detector was placed over the undisturbed opposite tibia. During 30 min of washout the count rate from the second probe did not become distinguishably greater than background, indicating that injected I-Ap was greatly diluted and that the effects of recirculation were negligible.

Isotope in marrow—Ideally, for measurement of blood flow in cortical bone, gamma emissions should come only from cortical bone, but inevitably some come from the marrow in spite of our choice of low-energy ^{125}I instead of ^{131}I . Previous experiments performed in this laboratory using xenon 133, which is very fat-soluble, as the indicator had shown that such a large percentage of the xenon resided in the marrow that it could not be used for estimation of cortical blood flow. The problem is much less with I-Ap because both its fat solubility and its energy of emission (0.0352 Mev vs. 0.0809 Mev for xenon) are less. Nineteen dogs were prepared as described for I-Ap washout. After indicator injection, four dogs were killed at the time of the peak count rate, $C^*(0)$, four dogs at 2 min, five at 7.5 min, and six at 15 min after injection. The tibias were removed, and a cortical window 6×1 cm was cut in the tibia anteriorly and then replaced. The radioactivity of both the bone and the marrow were measured externally with the position of the counter the same as when determining I-Ap washout curves. The fatty marrow then was scraped out so that macroscopically the tibia was free of marrow, the cortical window was replaced, and the radioactivity was recorded again. The count rates after removal of marrow were 70% of the count rates of the intact bone (Table 2).

In a separate series of nine experiments to evaluate the fraction of isotope absorbed by the overlying bone, tibias were removed from adult dogs that had just been killed but had not been used in any experiments with isotopes. A cortical window, 6×1 cm, was removed and the marrow was scraped out cleanly. A Gelfoam sponge was shaped to fit the cavity, evenly soaked with labeled I-Ap, and placed in the marrow cavity. Counts were then recorded with the cortical bone window in place and removed. The position and geometry of the bone and external counter were the same as for I-Ap washout experiments. The overlying cortical bone absorbed 40.5 ± 2.0 % of the emitted radiation from the isotope-containing sponge.

Perfusion of tibia via nutrient artery—In four dogs the nutrient artery was cannulated and perfused with the dog's own blood at a known constant rate with a variable rate pump (Extracorporeal Medical Specialties Co., Inc., Mount Laurel, N.J.). A fine needle was inserted into the cannula just proximal to its entry into the nutrient artery and $15 \mu\text{C}$ of I-Ap in 1 ml of isotonic saline were rapidly injected as described above. Washout curves were determined as before and cortical bone blood flow was calculated by equation 1. Eight separate perfusions were performed. The dogs were killed and the volume of whole tibia was determined by displacement of millet seed. It was then possible to compare perfusion rates of whole tibia with I-Ap estimations of cortical bone blood flow.

Results

Tibial Diaphyseal Blood Flow

Thirty-two washout curves from 10 dogs are shown in Fig. 4 as semilogarithmic plots of $C^*(t)/C^*(0)$ versus $f' \cdot t$. Equation 1 was used to calculate f' so that $f' \cdot t$ is the volume of blood per milliliter of bone estimated to have flowed out of the bone by time t . Flow estimates by the two methods did not differ significantly (Table 3).

Residue Function

Using equation 1 with a recording duration, T , of 15 min, the mean flow f' was 0.0152 ml/ml bone per min (SD , ± 0.003); mean f was 0.75 ml/100 g bone per min (SD , ± 0.15). In the nine cases in which T was 30 min, at which time $C^*(T)/C^*(0)$ averaged $6.2 \pm 2.8\%$, estimates of f' changed insignificantly from 0.0170 ml/ml bone per min (SD , ± 0.003) at 15 min to 0.0166 ml/ml bone per min (SD , ± 0.003) at 30 min.

Two-Exponential Analysis

Five of the curves were nearly monoexponential but 27 showed two components. Using equation A-8 or A-9 to fit the 27 curves, with $\rho_1 = \rho_2 = 1.94$ g/ml and $\lambda_1 = \lambda_2 = 0.15$, and equation 2 or A-12 or A-14 to calculate flow, we obtained a mean f' of 0.0145 ml/ml bone per min (SD , ± 0.0035) (Table 3). (The coefficients and rate constants of equation A-1 can be calculated by using the equations in the appendix.)

Figure 5 summarizes the exponential analysis by fitting the best two-exponential curve to the normalized plots of the 27 curves. On the average, 22% (SD , ± 7.4) of the indicator traversed a small high-flow region ($f_1' = 0.265$ ml/ml bone per min; SD , ± 0.194) and the remaining 76% (SD , ± 7.1) of the indicator went to bone with a relatively low flow ($f_2' = 0.0118$ ml/ml bone per min; SD , ± 0.003). The mean w_1 calculated from the individual curves was 0.0183 (SD , ± 0.0124).

Washout With I-Ap Injected Via Needle

Flow estimates did not differ from those obtained when the indicator was injected via a cannula. For seven washout curves in five dogs, mean f' calculated by equation 1 was 0.0141 ml/ml bone per min (SD , ± 0.003).

Effect of Nutrient Artery Cannulation on ^{85}Sr Clearance

In five dogs, the ratios of 10-min ^{85}Sr deposition in the tibia with cannulated nutrient artery to that in the opposite tibia (intact leg) were 1.31, 1.03, 1.17, 1.57, and 1.0. The mean values based on ^{85}Sr clearance were 3.83 ml/100 g bone per min (SD , ± 0.81) on the cannulated side and 3.23 ml/100 g bone per min (SD , ± 0.86) on the intact side ($0.1 < P < 0.2$). The mean value from 25 previous determinations on intact legs in dogs was 3.38 ml/100 g bone per min (SD , ± 1.0).

Because more ^{85}Sr was deposited in the tibia with the nutrient artery cannula than in the intact leg, it is clear that tibial diaphyseal flow was not decreased by the procedure.

Perfusion of Tibia at Known Rate of Flow

In a 17.8-kg dog with the nutrient artery perfusion set at 0.30 ml/min for two runs and the volume of tibia estimated to be 44.3 ml, the estimated pumped perfusion would be 0.0068 ml/ml whole tibia per min; the corresponding estimates were 0.0050 and 0.0052 ml/ml cortical bone per min by external monitoring of I-Ap washout and use of equation 1. Similarly, in a 15.4-kg dog with an estimated whole tibial volume of 37.0 ml, the pumped arterial flow was estimated to be 0.0271 and 0.0351 ml/ml whole tibia per min, while the residue function technique gave values of 0.0165 and 0.0222 ml/ml cortical bone per min. In a third dog (24 kg), the pumped arterial flow was 0.0208 ml/ml whole tibia per min, while the residue function technique gave 0.0170 ml/ml cortical bone per min. In a fourth dog (18.8 kg), pumped perfusions were 0.0118, 0.0088, and 0.0133 ml/ml whole tibia per min, while the residue function method gave values of 0.0103, 0.0054, and 0.0101 ml/ml cortical bone per min. These estimates of cortical bone blood flow determined by washout of I-Ap are $72.3 \pm 3.7\%$ of the value for pumped perfusion of whole tibia and agree with the

proportion of count rates after removal of marrow, 70%. The values for pumped perfusion are directly correlated with the values of cortical blood flow estimated by I-Ap washout ($r = 0.972$; $P < 0.001$).

Discussion

Analytical Approach

Equation 1 has been modified from that of Zierler (27). The original formula would be $f = \lambda \cdot C^*(0) / [\rho \cdot \int_0^T C^*(t) dt]$ if the experiment could only be carried to time T instead of to time infinity as theoretically required. Under such circumstances the integral in the denominator is too small and results in overestimates of flow. Subtraction of $C^*(T)$ from $C^*(0)$ in the numerator, as in equation 1, decreases the size of the numerator also and thereby introduces a correction which gives estimates closer to the correct flow than would otherwise be obtained. Equation 1 becomes identical to Zierler's formula when T is infinity; when $C^*(t)$ is monoexponential, equation 1 provides an analytically exact estimate of f . In practice, equation 1 may provide overestimates or underestimates of f depending on the slope of $C^*(t)$, but the errors are less than with the original formula (with integration to T only) which always produces overestimates. The observed insensitivity of the estimates of f to T (15 or 30 min) confirms in a practical way the intuitive theoretical argument. These observations are similar to those shown in the left lower panel of Fig. 6 of an earlier study (1) on xenon washout from the heart, in which equation 1 was applied exactly as it has been applied here.

The use of equation 1 depends on the assumption that no tracer leaves the field of the detector before all has entered. Since the duration of injection was less than 5 sec and peak counts were obtained in less than 20 sec (avg = 13 sec) after the start of the injection and since appearance times in the venous outflow are probably greater than 15 sec and, in the short time involved, can result only in a very small loss of indicator, we believe that the assumption that the peak count rates are a measure of the total injected dose is reasonable.

Equation 2 and the appendix are formulated as if the blood flowed through two independent, wholly mixed regions in parallel. There is no anatomic or physiologic basis for this (especially since marrow washout was seen to have a time course similar to that of bone) and, therefore, it is reasonable to consider ρ_1 and ρ_2 equal to the average ρ and λ_1 and λ_2 equal to the average λ . When these conditions hold, then two-exponential analysis becomes simply a method of fitting or describing the curve, equivalent to finding the area in equation 1, and the values for f_1, f_2, w_1 and w_2 are the descriptors. Washout curves from organs generally are "multiexponential"—meaning only that the fractional escape rate of contained tracer decreases continuously as must occur with any heterogeneity of flow or of regional volume of distribution (partition coefficient). Thus, in applying two-exponential analysis we do not suppose that there are only two residue functions, nor do we think that using more exponentials would provide more information from these curves. Since the curves have only been recorded for 30 min, it is likely that slower components of washout would be seen by recording for longer periods but their presence would have little influence on either method of estimating flow.

Flow Limitation to I-Ap Washout

The estimation of flow by equation 1 or 2 does not require that the washout must be completely flow-limited, i.e., that diffusional barriers do not impede transport from tissue to blood or vice versa. However, the values of ρ and λ in equation A-8 would not have been appropriately chosen in this analysis if washout were not flow-limited, and residue function analysis would be less accurate because of the prolongation of the tails. Fortunately, I-Ap

blood-bone exchange does appear to be flow-limited. The washout curves were obtained at different flows and yet were similar in shape when plotted as $C^*(t)/C^*(0)$ against $f't$ (Fig. 4), just as occurs in the heart (26). This conclusion is not vitiated by the fact that the abscissa of Fig. 4 was based on the flow estimates derived from the curves (if the flow had been measured independently, further argument would be obviated). The additional arguments are as follows.

1. The volume of distribution of I-Ap is far larger than the vascular space. Figure 2 shows that for I-Ap there apparently is an equilibrium between bone and outflowing venous blood even when A-V concentration differences are large. Figure 6 illustrates this important fact in a different way—the partition coefficient is not systematically lower at high A-V differences over a range from 0 to 45% and, therefore, there is no diffusion limitation to the exchange.
2. If washout of isotope that has diffused into tissue were even partially diffusion-limited, then an increase in flow would produce a less than proportional increase in rate of washout, and because the proportionate change would not be the same in all regions there would be changes in shape of the curve as well. However, flow values varied from dog to dog and varied in the same dog on repeated observations, but flow affected only the timing of the washout curves. The shapes of all the curves were similar when considered as a function of the volume (f' times t) that had passed through the organ.
3. If there were a diffusion limitation to washout, there also would be one for movement into the tissue, and the amount taken up in the first passage would diminish as flow increased. This would show up in the two-exponential fitting as an increase in w_1 with increasing flow. But values for w_1 showed a random relationship to f_1' and f' (Table 3) as did values of w_2 to f_2' and f' ; so it can safely be concluded that there was no diffusion limitation to washout.

Estimation of f' , f_1' , and f_2' is dependent on knowledge of the partition coefficient (λ) for I-Ap in bone. The value, in these experiments, of 0.26 g of water removed per 1 ml of bone (or 26 vol%) at 100 C is near to that reported by Biltz and Pellegrino (2) and Robinson (20), as is the specific gravity of 1.94. In the second series of seven dogs (Table 1), the weight loss when bone was dried to a constant weight at 50 C averaged 0.149 g/ml (or 14.9 vol%), nearly identical with Robinson's estimate of the cellular-osteocyte water space of bone (12–15 vol%). Since this is so close to our estimate of λ , it suggests that I-Ap may distribute throughout this space, i.e., I-Ap distributes itself in the intracellular and extracellular water space of cortical bone as it does in other tissue (12,22). The water removed at 100 C may have been bound in collagen of bone.

Source of Recorded Emissions

It may be only coincidental that marrow counts remained constant at 30% of the total counts for 15 min of washout. By virtue of this observation, marrow counts do not interfere with the estimate of cortical bone blood flow. It may also mean that the marrow blood flow has the same f'/λ as in the bone, suggesting therefore that the marrow blood flow is about $0.0152 \times 0.63/0.150$ or 0.063 ml/ml marrow per min, a value close to values directly determined for adipose tissue of the dog by Oro and co-workers (19). (In separate studies we have determined that partition coefficient for diaphyseal marrow, an adipose tissue, to be 0.63.) If the f'/λ for marrow were rather different, then the externally recorded washout curves would have shown more components and correction would have been necessary.

However, although absorption of emissions by the bone has the advantage of reducing the sensitivity to tracer in the marrow, it also means that tracer in the deeper layers of the cortex

has less influence than that in external layers. This is not too important because the volume of the internal layers is less (in proportion to the square of the distance from the bone axis) and because there is little reason to expect superficial and deep layers of cortex to have very different flows since the capillary beds of cortical bone anatomically are uniform (16).

Normal or Collateral Flow?

In the experiments with the cannulated nutrient artery, through which there is no flow after the tracer injection, what is measured is collateral flow to the tibial diaphysis from epiphyseal-metaphyseal arteries and periosteal vessels. In spite of the cannulation, the flow apparently is normal, at least as measured by ^{85}Sr deposition. Strontium extraction during transcapillary passage is less at higher flows than at lower flows (3) and, therefore, the observation that 20% more ^{85}Sr is deposited in the bone with the cannulated artery may mean that the flow was 30% higher. An increase in ^{85}Sr deposition after cannulation is somewhat unexpected but greatly reassuring that the flow estimates do represent flows no less than normal.

Equally reassuring are the observations that flows estimated with needle injections which do not obstruct the nutrient artery were the same as those estimated with a cannula in place.

These two experiments show that collateral communications between diaphysis and the ends of the long bone are excellent in the mature dog's tibia and that our estimates are of essentially normal flows.

Comparison With Flow Estimate by Others

The values for f' (ml/ml bone per min) reported here are in agreement with estimates reported by Edholm and co-workers (10) based on plethysmography.

Our approach, and that of Zierler's, is based on conservation of matter and theoretically must be correct. If there are errors due to not observing a long low tail of the washout curves, then our values are too high, not too low. It is difficult to suppose that, for experimental reasons, our estimates are many times too low, because the ^{85}Sr experiments gave values for control mature dogs similar to those obtained previously (21). The question now is how to reconcile the present estimates with the much higher values obtained by others.

Other previous reports are now considered. One can estimate experimentally both the fractional volume occupied by erythrocytes and their mean transit time, $\bar{t}$. The volume/time ratio gives the flow (ml/ml bone per min), illustrating that the method is conceptually similar to ours. Assuming $\rho = 2.0$ g/ml, Brookes (4) obtained values of 0.34–0.58 ml/ml bone per min and White (25) obtained a mean of 0.32 ml/ml bone per min. However, in both studies, $\bar{t}$ was calculated from the fastest initial slope, which resulted in overly small estimates (10 sec or less). Such small values of $\bar{t}$ correspond to those of our fast component: using equation A-10 and f' in Table 3, then $\bar{t}_1 = 1/k_1 = \lambda_1/\rho_1 f_1 = \lambda_1/f_1'$. An average $\bar{t}_1$ for antipyrine is 0.150×60 (sec/min)/ $0.265 \text{ min}^{-1} = 34$ sec. Since I-Ap volume of distribution in bone is approximately three to four times the erythrocyte space (25), the value of 34 sec is quite comparable to their estimate of 10 sec and suggests that their neglect of slower components of erythrocyte washout resulted in underestimates of mean transit time and overestimates of flow.

Soluble tracer extraction techniques can be used, as in the Kety-Schmidt technique; again λ must be known. But the clearance techniques of Weinman et al. (24), Copp and Shim (6), and Van Dyke et al. (23) are not so clearly based on conservation of material since they were not able to record concentrations in the nutrient vein simultaneously. However, their estimates of flow may not be so different from ours as first appears. For example, in the

whole tibia, apparently including marrow, Copp and Shim (6) obtained values of 0.1 ml/g per min; if the marrow weight were 10% of the total weight but, as our results suggest, the flow four times as high as in bone and if the early extraction were as high in marrow as in bone (which is plausible because of protein binding in the first seconds or minutes), then the estimated cortical flow would be decreased to 0.069 ml/g cortical bone. If the bone were 20% marrow whose flow was seven times cortical flow, the estimated cortical flow would be 0.036 ml/g per min. These estimates of cortical flow are probably still too high because they are based on estimates of those found by Cumming (7)—mean of 0.51 ml/g marrow per min with a range of 0.27–1.1 ml/g per min in the rabbit femur where the ratio of bone weight to marrow weight averaged 5.3 ± 1.3 . However, his marrow flow estimates must be somewhat too high because what he actually measured was total flow in the nutrient vein of the rabbit femur and then, on the basis that the cortical bone usually was only slightly stained after injection of Evans blue in the nutrient artery, he assumed that a negligible amount of flow went to cortical bone. It must be recognized that our values are for cortical bone, that Copp and Shim studied mixed bone and marrow (whole tibia), and that Cumming was interested primarily in erythroid marrow of the rabbit. Michelson (18) has shown a 15-fold variation in flow in rabbit marrow depending on the hemopoietic activity of the marrow; this further emphasizes the necessity of evaluating the type of marrow tissue one is dealing with when measuring bone blood flow. By combining Cumming's total flow estimates and ratio of bone to marrow weights and Copp and Shim's clearances, we obtain approximate estimates of 0.04 ml/g bone per min for cortical flow and 0.4 ml/g marrow per min for marrow flow, values which are not dramatically different from ours.

Deposition of ^{42}K and microspheres was used by Kane and Grim (14) and deposition of labeled particles, by Brookes (5) who stated that 27.5% of cardiac output flows to the skeleton. The ^{42}K study (14) also implied that a large percentage of cardiac output goes to the skeleton and showed that cortical bone blood flow exceeded skeletal muscle flow by a factor of 2.8. The values appear unreasonably high in view of the low turnover of tibial cortical bone of the mature dog, i.e., the forming of new bone and resorbing of old bone (21). Kane (13) made one observation of ^{42}K deposition in a human leg just prior to hindquarter amputation and obtained a value of 0.01 ml/g per min for cortical bone blood flow, quite similar to our estimates. No doubt, the deposit time for tracer exceeded 60 sec since hindquarter amputation would take more than 1 min. With deposition techniques, the amount of an extractable solute deposited depends on the timing of the sudden stoppage of the circulation and on the different numbers of recirculations through different organs. Also the microsphere deposition is influenced by intravascular streaming and plasma skimming. Domenech et al. (9) found that microsphere size influenced the distribution throughout the myocardium.

Acknowledgments

The authors are grateful to Mr. Glenn Christensen who assisted with the experiments.

This investigation was supported in part by Research Grants AM-8658, HE-9719, and FR-7 from the National Institutes of Health.

Appendix

External Monitoring: Equations for Two Compartments in Parallel

Application of two-compartment analysis to the estimation of organ blood flow is based on the assumption that the organ consists of two instantaneously and completely mixed pools which are separate from each other and through which the flow is steady. The equation has the general form:

$$C^*(t) = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} \quad (\text{A-1})$$

in which the sum of the intercepts A_1 and A_2 is $C^*(0)$, and k_1 and k_2 are rate constants. The rate constants are calculated from each of the lines on semilog paper. If $C_1^*(t)$ is the steepest line, then $k_1 = \log_e (C^*(t_1)/C^*(t_2))/(t_2 - t_1)$ in which the times t_1 and t_2 are arbitrary. The following derivation is given in detail so that interpretation of the rate constants and, more particularly, of the coefficients can be made completely clear. The following symbols are used; some equivalent expressions are given now but will be proved later:

t = time (min)

m = mass of indicator injected (g); $m_i(t)$ = mass of residual indicator in tissue i (where $i = 1$ or 2) at time t . $m(t) = m_1(t) + m_2(t)$ = total residual indicator in tissue.

V = total volume of organ (ml) = $V_1 + V_2$

V_i = volume of tissue i (ml)

v_i = relative volume of tissue $i = V_i/(V_1 + V_2) = V_i/V$

V_{Di} = volume of distribution (ml) in tissue $i = \lambda_i V_i$

v_{Di} = relative volume of distribution in tissue $i = V_{Di}/(V_{D1} + V_{D2})$

M = mass of organ (g)

M_i = mass of tissue i (g) = $\rho_i V_i = \rho_i V_{Di}/\lambda_i$

w_i = relative mass of tissue $i = M_i/M$

ρ = density of tissue (g/ml) = M/V

ρ_i = density of tissue i (g/ml) = M_i/V_i

λ_i = partition coefficient for tissue i = solubility of indicator in tissue i /solubility of indicator in blood

λ = average partition coefficient for organ = $(\lambda_1 V_1 + \lambda_2 V_2)/V$

F = flow (ml/min) to whole organ = $F_1 + F_2$

f = flow (ml/g organ per min) = $F/(\rho V) = f'/\rho$

f' = flow (ml/ml organ per min) = F/V

f'' = flow (ml/ml volume of distribution in whole organ per min) = $F/(V_{D1} + V_{D2})$

F_i = flow (ml/min) to tissue i

f_i = flow (ml/gm of tissue i per min) = $F_i/\rho_i V_i = f'_i/\rho_i$

f'_i = flow (ml/ml of tissue i per min) = $F_i/V_i = F_i \lambda_i / V_{Di} = \lambda_i f''_i$

f''_i = flow (ml/ml volume of distribution in tissue i per min) = F_i/V_{Di}

τ_i = exponential time constant (min) of i th component = $V_{Di}/F_i = 1/f''_i = \lambda_i/(\rho_i f_i)$

Tissue is defined as consisting of blood plus extravascular structures. This implies that the partition coefficient λ is defined not in terms of the ratio of extravascular concentration to intracapillary blood concentration but as the ratio of the average concentration in the tissue (including contained blood) to the concentration in the outflowing blood.

An external scintillation detector is used to record the residual activity $C^*(t)$ within the organ. Assuming the injection to be instantaneous, so that the activity $C^*(0)$ was maximal, at time zero, immediately after injection into the inflow and assuming that the efficiency of detection was constant throughout the experiment, then the residue function $H^*(t)$, the fraction of injected indicator still residing in the organ at time t , is $C^*(t)/C^*(0)$. In these experiments this assumption is quite reasonable. Furthermore, it is assumed that the indicator is sufficiently diffusible so that there is always a complete equilibration between capillary blood and each tissue locally (the ratio of tissue concentration to blood concentration is always the partition coefficient). This actually is equivalent to saying that the washout of indicator is purely flow-limited and is not slowed by diffusional resistances.

Then, the rate of mass loss from tissue i is

$$\frac{dm_i(t)}{dt} = -\frac{F_i}{V_{D_i}} \cdot m_i(t) = -f_i'' m_i(t) \quad (\text{A-2})$$

which on integration and substitution of the initial condition is:

$$m_i(t) = m_i(0)e^{-f_i'' t} \quad (\text{A-3})$$

Now, assuming mixing at the injection site, the injected mass m flows with the blood and is split into two fractions in proportion to the relative flows to each tissue:

$$\frac{m_i(0)}{m} = \frac{F_i}{F} \quad (\text{A-4})$$

and

$$\begin{aligned} m(t) &= m_1(t) + m_2(t) \\ &= m_1(0)e^{-f_1'' t} + m_2(0)e^{-f_2'' t} \end{aligned} \quad (\text{A-5})$$

The residual fraction of injected indicator, $H^*(t)$, is $m(t)/m$; therefore, the last two equations (3 and 4) can be combined and rephrased:

$$H^*(t) = \frac{F_1}{F} e^{-f_1'' t} + \frac{F_2}{F} e^{-f_2'' t} \quad (\text{A-6})$$

Substituting $F_1 = f_1'' V_{D_1}$, $F = F_1 + F_2$, and $f'' = F/(V_{D_1} + V_{D_2})$ gives:

$$H^*(t) = \frac{f_1'' V_{D_1}}{f'' (V_{D_1} + V_{D_2})} \cdot e^{-f_1'' t} + \frac{f_2'' V_{D_2}}{f'' (V_{D_1} + V_{D_2})} \cdot e^{-f_2'' t}$$

or

$$H * (t) = v_{D_1} \frac{f_1''}{f''} e^{-f_1'' t} + v_{D_2} \frac{f_2''}{f''} e^{-f_2'' t} \quad (\text{A-7})$$

Although this form provides a simple interpretation of the rate constants, flow per unit volume of distribution, the coefficients $v_{D_i} f_i''/f''$ are not readily deciphered. Use of different units for flow, as for f' or f , does not complicate the rate constants very much and does make the coefficients simpler. First, substituting f_i'/λ_i for f_i'' and f'/λ for f'' , which are related via the definition, $\lambda = (\lambda_1 V_1 + \lambda_2 V_2)/V$, gives:

$$H * (t) = \frac{\lambda v_{D_1} f_1'}{\lambda_1 f'} \cdot e^{-f_1' t/\lambda_1} + \frac{\lambda v_{D_2} f_2'}{\lambda_2 f'} \cdot e^{-f_2' t/\lambda_2}$$

and, since

$$\frac{\lambda v_{D_i}}{\lambda_i} = \frac{\lambda V_{D_i}}{\lambda_i (V_{D_1} + V_{D_2})} = \frac{\lambda \lambda_i V_i}{\lambda_i \lambda V} = \frac{V_i}{V} = v_i$$

then

$$H * (t) = \frac{v_1 f_1'}{f'} \cdot e^{-f_1' t/\lambda_1} + \frac{v_2 f_2'}{f'} \cdot e^{-f_2' t/\lambda_2} \quad (\text{A-8})$$

If one also takes into account the density of the tissue ρ_i , then $f_i \rho_i$ may be used for f_i' and $f \rho$ for f' :

$$H * (t) = \frac{v_1 \rho_1 f_1}{\rho f} \cdot e^{-\rho_1 f_1 t/\lambda_1} + \frac{v_2 \rho_2 f_2}{\rho f} \cdot e^{-\rho_2 f_2 t/\lambda_2}$$

But $v_1 \rho_1/\rho = V_1 \rho_1/V \rho = M_1/M = w_1$ the relative mass of tissue 1, and therefore:

$$H * (t) = \frac{w_1 f_1}{f} \cdot e^{-\rho_1 f_1 t/\lambda_1} + \frac{w_2 f_2}{f} \cdot e^{-\rho_2 f_2 t/\lambda_2} \quad (\text{A-9})$$

This equation was used previously for calculating coronary blood flow (1) but the f was omitted.

The sum of the coefficients is necessarily 1.0 and f can be calculated from the estimates of the rate constants and intercepts obtained by fitting equation A-9 to the experimental data. The rate constants k_1 and k_2 and the intercepts A_1 and A_2 are interpreted as follows:

$$k_1 = \rho_1 f_1 / \lambda_1$$

and therefore:

$$f_1 = \lambda_1 k_1 / \rho_1 \quad (\text{A-10})$$

Similarly,

$$f_2 = \lambda_2 k_2 / \rho_2$$

where λ_i , ρ_i , and k_i are shown. Now,

$$A_1 = w_1 f_1 / f \text{ and } A_2 = 1.0 - A_1$$

and

$$\frac{w_1 f_1 A_1}{w_2 f_2 A_2}$$

Therefore:

$$w_1 = \frac{A_1}{1 - A_1} \cdot (1 - w_1) \frac{f_2}{f_1} = \frac{A_1 f_2}{(1 - A_1) f_1 + A_1 f_2} \quad (\text{A-11})$$

and

$$w_2 = 1 - w_1$$

so that the average organ blood flow per gram of tissue, f , can be calculated from the f_i and w_i by:

$$f = w_1 f_1 + w_2 f_2 \quad (\text{A-12})$$

If one desires to use equation A-8, in terms of flow per unit volume, or cannot use equation A-9, because the densities are not known, then the fractional volumes, v_i , can be derived analogously to equations A-11 and A-12, thus providing the v_i and the average flow f' :

$$v_1 = A_1 f'_2 / (A_2 f'_1 + A_1 f'_2) \quad (\text{A-13})$$

and

$$v_2 = 1.0 - v_1$$

and

$$f' = v_1 f'_1 + v_2 f'_2 \quad (\text{A-14})$$

References

1. Bassingthwaite JB, Strandell T, Donald DE. Estimation of coronary blood flow by washout of diffusible indicators. *Circulation Res.* 1968; 23:259–278. [PubMed: 4874081]
2. Biltz RM, Pellegrino ED. The chemical anatomy of bone. I. A comparative study of bone composition in sixteen vertebrates. *J Bone Joint Surg.* 1969; 51a:456–466. [PubMed: 4976035]
3. Bosch WJ. Plasma ^{45}Ca clearance by the tibia in the immature dog. *Am J Physiol.* 1969; 216:1150–1157. [PubMed: 5797296]
4. Brookes M. Blood flow rates in compact and cancellous bone, and bone marrow. *J Anat.* 1967; 101:533–541. [PubMed: 6051732]
5. Brookes M. Arteriolar blockade: a method of measuring blood flow rates in the skeleton. *J Anat.* 1970; 106:557–563. [PubMed: 5423944]
6. Copp DH, Shim SS. Extraction ratio and bone clearance of Sr^{85} as a measure of effective bone blood flow. *Circulation Res.* 1965; 16:461–467. [PubMed: 14289155]
7. Cumming JD. A study of blood flow through bone marrow by a method of venous effluent collection. *J Physiol, London.* 1962; 162:13–20. [PubMed: 13882611]
8. Defares, JG.; Sneddon, IN. *An Introduction to the Mathematics of Medicine and Biology.* Chicago: Year Book; 1960. p. 582
9. Domenech RJ, Hoffman JIE, Noble MIM, Saunders KB, Henson JR, Subijanto S. Total and regional coronary blood flow measured by radioactive microspheres in conscious and anesthetized dogs. *Circulation Res.* 1969; 25:581–596. [PubMed: 5351326]
10. Edholm OG, Howarth S, McMichael J. Heart failure and bone blood flow in osteitis deformans. *Clin Sci.* 1945; 5:249–260. [PubMed: 21011941]
11. Frederikson A, Honour AJ, Copp DH. Measurement of initial bone clearance of Ca^{45} from blood in the rat (Abstract). *Federation Proc.* 1955; 14:49.
12. Huckabee WE. Use of 4-aminoantipyrine for determining volume of body water available for solute dilution. *J Appl Physiol.* 1956; 9:157–162. [PubMed: 13376421]
13. Kane WJ. The determination of blood flow to tissues of the human leg (Abstract). *J Bone Joint Surg.* 1968; 50a:1070.
14. Kane WJ, Grim E. Blood flow to canine hind-limb bone, muscle, and skin: a quantitative method and its validation. *J Bone Joint Surg.* 1969; 51a:309–322. [PubMed: 5767322]
15. Kelly PJ. Measurement of oxygen saturation in arterial and venous blood of bone in normal puppies and in puppies with arteriovenous fistula. *Proc Staff Meetings Mayo Clinic.* 1966; 41:95–102.
16. Kelly PJ, Janes JM. Microangiographic and histologic studies of vascular anatomy of the femur and tibia distal to femoral and iliac arteriovenous fistulas in dogs. *Anat Record.* 1968; 162:255–267. [PubMed: 5702214]
17. Krasnow N, Levine HJ, Wagman RJ, Gorlin R. Coronary blood flow measured by I-131 iodoantipyrine. *Circulation Res.* 1963; 12:58–62. [PubMed: 14035508]
18. Michelson K. Hemodynamics of the bone marrow circulation. *Acta Physiol Scand.* 1968; 73:264–280. [PubMed: 5709584]
19. Oro L, Wallenberg L, Rosell S. Circulatory and metabolic processes in adipose tissue *in vivo*. *Nature.* 1965; 205:178–179. [PubMed: 14276269]

20. Robinson, RA. Chemical analysis and electron microscopy of bone. In: Rodahl, K.; Nicholson, JT.; Brown, EM., Jr, editors. Bone as a Tissue. New York: McGraw; 1960. p. 186-250.
21. Sim FH, Kelly PJ. Relationship of bone remodeling, oxygen consumption, and blood flow in bone. *J Bone Joint Surg.* 1970; 52a:1377–1389. [PubMed: 5469192]
22. Soberman R, Brodie BB, Levy BB, Axelrod J, Hollander V, Steele JM. The use of antipyrine in the measurement of total body water in man. *J Biol Chem.* 1949; 179:31–42. [PubMed: 18119217]
23. Van Dyke D, Anger HO, Yano Y, Bozzini C. Bone blood flow shown with F^{18} and the positron camera. *Am J Physiol.* 1965; 209:65–70. [PubMed: 14343770]
24. Weinman DT, Kelly PJ, Owen CA Jr, Orvis AL. Skeletal clearance of Ca^{47} and Sr^{85} and skeletal blood flow in dogs. *Proc Staff Meetings Mayo Clinic.* 1963; 38:559–570.
25. White NB, Ter-Pogossian MM, Stein AH Jr. A method to determine the rate of blood flow in long bone and selected soft tissues. *Surg, Gynecol Obstet.* 1964; 119:535–540. [PubMed: 14210152]
26. Yipintsoi T, Bassingthwaighe JB. Circulatory transport of iodoantipyrine and water in the isolated dog heart. *Circulation Res.* 1970; 27:461–477. [PubMed: 5452741]
27. Zierler KL. Equations for measuring blood flow by external monitoring of radioisotopes. *Circulation Res.* 1965; 16:309–321. [PubMed: 14270567]

Fig. 1. Modification of height/area method for estimation of bone blood flow from I-Ap washout. $f' = 0.0124$ ml/ml bone per mm in this example (see eq 1).

Fig. 2.

External counts in first 10 perfusion experiments. $C^*(\infty)$ is taken as final count rate obtained by external detector over bone multiplied by final ratio of tracer concentration in inflow perfusate to that in nutrient vein outflow.

Fig. 3.
External counts in second seven perfusion experiments; in five, a true steady state existed.

Fig. 4. Thirty-two washout curves from 10 dogs, $f' \cdot t$ is the volume of blood per milliliter of bone which has left the bone in the time since arbitrary zero time. Curves at different flow rates are shown by different types of line.

Fig. 5.
Twenty-seven washout curves averaged and fitted with a two-exponential equation.

Fig. 6.
Relationship of partition coefficient to perfusate (C_A)-venous (C_V) difference.

Table 1

Determination of partition coefficient

Bone Activity, 10 ³ counts/sec per ml	Venous Blood Activity (V), 10 ³ counts/sec per ml	Partition Coef (λ)	Arterial Perfusate Activity (A), 10 ³ counts/sec per ml	(C _A –C _V)/C _A , %	Wt Loss, g/ml		λ/Wt Loss		Specific Gravity	Perfusion, min
					50 C	100 C	50 C	100 C		
Infusion of I-Ap in blood at 2.2 ml/min										
0.86	7.13	0.121	7.63	6.6		0.283		0.427	2.02	8
1.23	8.17	0.151	8.86	7.8		0.289		0.522	1.89	12
0.91	6.69	0.136	4.40	9.5		0.256		0.531	2.08	19
1.27	7.50	0.170	8.00	6.2		0.417		0.408	2.15	12
1.10	4.91	0.224	7.97	38.4		0.330		0.679	2.10	18
1.14	6.98	0.163	7.83	10.8		0.186		0.876	1.83	18
0.57	4.23	0.136	7.57	44.1		0.260		0.523	1.91	11
1.50	8.58	0.175	9.58	6.2		0.220		0.795	1.78	10
1.53	8.70	0.176	9.20	5.4		0.423		0.416	1.85	10
0.95	7.29	0.130	12.23	40.0		0.198		0.656	1.90	16
Infusion of I-Ap in blood at 1.5 ml/min										
1.94	15.76	0.123	15.75	0	0.128	0.203	0.961	0.606	1.95	17
2.39	17.27	0.138	17.27	0	0.149	0.210	0.912	0.647	1.98	16
2.65	14.45	0.183	16.88	14.4	0.173	0.226	1.057	0.809	1.90	15
2.52	18.04	0.140	17.98	0	0.158	0.219	0.886	0.640	1.92	32
2.23	15.79	0.140	15.80	0	0.149	0.218	0.939	0.660	1.94	46
2.02	15.96	0.126	16.00	0	0.152	0.212	0.830	0.610	1.89	30
1.72	13.70	0.126	15.66	14.3	0.132	0.206	0.926	0.630	1.91	10
Mean ± SD		0.150 ± 0.027			0.149 ± 0.015	0.256 ± 0.070	0.930 ± 0.070	0.614 ± 0.135	1.94 ± 0.10	

Table 2
Effect of isotope in marrow on externally recorded activity

Time after Injection, min	No. of Obs	Activity, Mean $\pm$ SD, (Without Marrow/With Marrow) $\times$ 100
0	4	70.7 $\pm$ 9.2
2	4	68.6 $\pm$ 6.5
7.5	5	69.0 $\pm$ 5.9
15	6	70.5 $\pm$ 5.9

Table 3

Estimates of tibial blood flow

Dog No.	Exp No.	Flow by Two Methods of Data Analysis				
		Residue function f' by eq 1	Two-exponential analysis by eq 2			
			f_1'	f_2'	W_1^*	f' by modified eq 2 [†]
1	1	0.0106	0.334	0.0086	0.0024	0.0094
	2	0.0144		0.0136		0.0136
	3	0.0124		0.0134		0.0134
2	4	0.0119	0.268	0.0074	0.0119	0.0105
	5	0.0145	0.268	0.0095	0.0138	0.0141
	6	0.0120	0.173	0.0077	0.0138	0.0100
3	7	0.0150	0.318	0.0100	0.0088	0.0126
	8	0.0148	0.104	0.0087	0.0485	0.0133
	9	0.0200	0.131	0.0151	0.0365	0.0225
	10	0.0170	0.094	0.0124	0.0243	0.0144
	11	0.0209	0.152	0.0169	0.0267	0.0205
	12	0.0180	0.188	0.0175	0.0133	0.0197
4	13	0.0130		0.0138		0.0138
	14	0.0185	0.205	0.0117	0.0258	0.0174
	15	0.0181	0.282	0.0137	0.0136	0.0173
	16	0.0190	0.266	0.0157	0.0203	0.0207
5	17	0.0142	0.076	0.0107	0.0343	0.0130
	18	0.0110	0.060	0.0097	0.0180	0.0106
6	19	0.0213	0.222	0.0135	0.0316	0.0200
	20	0.0142	0.707	0.0120	0.0019	0.0138
7	21	0.0125	0.144	0.0102	0.0166	0.0124
	22	0.0206	0.322	0.0155	0.0118	0.0191
	23	0.0178	0.158	0.0126	0.0278	0.0170
	24	0.0150	0.619	0.0107	0.0051	0.0138
	25	0.0126	0.590	0.0105	0.0052	0.0136

Dog No.	Exp No.	Flow by Two Methods of Data Analysis				
		Residue function f' by eq 1	Two-exponential analysis by eq 2			
			f_1'	f_2'	w_1^*	f' by modified eq 2 [†]
8	26	0.0158	0.427	0.0120	0.0086	0.0154
	27	0.0114		0.0113		0.0113
9	28	0.0109	0.659	0.0080	0.0030	0.0100
10	29	0.0154		0.0151		0.0151
	30	0.0137	0.125	0.0081	0.0257	0.0111
	31	0.0133	0.195	0.0095	0.0062	0.0106
Mean ± SD	32	0.0155	0.074	0.0124	0.0390	0.0148
		0.0152 ± 0.003	0.265 ± 0.185	0.0118 ± 0.0027	0.0183 ± 0.0124	0.0145 ± 0.0035

Values are given in ml/ml bone per min.

* For monoexponential curves (exp 2, 3, 13, 27, and 29), w_1 = zero and w_2 = 1.0.

† See eq 13 in appendix.