Published in final edited form as:

J Bone Joint Surg Am. 1979 September ; 61(0): 857–866.

Extraction of Minerals after Experimental Fractures of the Tibia in Dogs*

SEAN P.F. HUGHES, M.S., F.R.C.S.[†], GERARD J. LEMON, F.R.C.S.[†], DAVID R. DAVIES, M.D., F.R.C.S.[†], JAMES B. BASSINGTHWAIGHTE, M.D.[†], and PATRICK J. KELLY, M.D.[†]
Mayo Clinic and Mayo Foundation, Rochester, Minnesota

Abstract

In a study of a standardized fracture of the tibia in dogs, we examined the relationship between extraction of strontium-85, as measured by a multiple indicator-dilution technique, and blood flow, as determined by an iodoantipyrine-washout technique. Although the blood flow at the fracture site increased from a control value of 1.5 milliliters per 100 grams of bone per minute to a value of 6.65 milliliters per 100 grams of bone per minute at two weeks, the maximum instantaneous extraction of ⁸⁵Sr by the fractured tibiae did not change from a value of about 0.77 of the amount injected. These results suggest that the increase in blood flow is accomplished by recruitment of capillaries, that the permeabilities of the capillary beds in the fractured tibia and in the normal tibia are similar, and that the pattern of flow in the fractured tibia remains non-uniform. The increased capillary surface area available for exchange is suggested as the reason that strontium extraction was unchanged despite the increased flow.

Clinical Relevance—This study has shown that calcium is only moderately rapidly exchangeable across the capillary membrane and made available for uptake by osteoblasts and deposition in callus at the fracture site. Because calcium delivery via capillary is essential, and because capillary surface area is ordinarily increased in the region of a healing fracture, we can now see clearly that clinical treatment, in particular manipulative intervention, should be carried out in a fashion that minimizes damage to the capillary bed. The exchange processes described in this study are the same as those involved in the deposition of agents used for bone-scanning, and thus this information provides a basis for the timing of scanning studies and for their interpretation.

The use of tracer kinetics provides an important technique for the study of the metabolic activity of tissues. Tracers that concentrate in bone, such as the isotopes ⁸⁵Sr and ⁴⁷Ca, have been helpful in examining exchange between circulating blood and bone. The quantity of tracer that enters bone must be related to the blood flow — that is, to the amount of tracer delivered to the bone — and to the diffusion of tracer through the capillary wall of the capillary tube. If bone is like other tissues, the flux of ions and solutes across the capillary wall is related to the flow of the fluid carrying the ions and solutes, to the extraction of ions in the tissue, to the permeability of the capillary wall, and to the surface area of the capillaries.

The quantity of tracer that enters bone can be expressed most simply as deposition for a given period of time. For example, if the period is ten minutes, deposition can be expressed by the formula:

*This investigation was supported in part by Research Grants AM-15980 and HL-9719 from the National Institutes of Health. Public Health Service. Dr. Hughes is the recipient of a grant from the Wellcome Trust.

Copyright 1979 by The Journal of Bone and Joint surgery, Incorporated

[†]200 First Street, S.W., Rochester, Minnesota 55901, Please address reprint requests to Dr. Hughes. c/o Section of Publications.

$$\text{Deposition} = \frac{B}{A}$$

where A represents average inflow concentration of tracer during ten minutes and B is the tracer content in bone. The units of A are counts per second per milliliter (counts s⁻¹ ml⁻¹) of blood and the units of B are counts per second per gram (counts s⁻¹ g⁻¹) of bone. Therefore,

$$\begin{aligned} {}^{85}\text{Sr deposition at 10 min} &= \frac{B(\text{counts s}^{-1}\text{g}^{-1})}{A(\text{counts s}^{-1}\text{ml}^{-1})} \cdot 10 \text{ min}^{-1} \\ &= \frac{\text{g}^{-1}}{\text{ml}^{-1}} \cdot 10^{-1} \\ &= \frac{\text{ml}}{\text{g}} \cdot \frac{1}{10 \text{ min}} \end{aligned} \quad \text{or}$$

Dividing deposition by the time of the experiment, ten minutes, gives clearance, which has the units milliliters per gram of bone per minute, the same units as those for blood flow in bone. However, clearance may also be viewed as the minimum volume of blood entering an organ per unit of time which is capable of supplying the amount of indicator removed per unit of time from the blood during its passage through the organ. Clearance is the product of flow multiplied by extraction, extraction being the fraction of the injected tracer removed from the blood during passage through the organ. Therefore,

$$\text{Blood flow} = \frac{\text{Clearance}}{\text{Extraction}}$$

Indicator-dilution techniques are very helpful in analyzing the complex interrelationships of deposition, clearance, extraction, and flow, because these methods allow measurement of extraction and estimations of permeability of the tissue capillary walls to ions and solutes. The canine tibia provides a good experimental model for such studies, because the nutrient artery is easily cannulated and tracers can be injected directly into the arteries leading to cortical-bone capillaries (Fig. 1). The venous blood leaving the bone can be collected from the ipsilateral femoral vein.

It is possible to account for 99 per cent^{4,10} of the injected tracer (⁸⁵Sr) in this system. An example of such an indicator-dilution curve is illustrated in Figure 2. The fraction of the injected dose of the tracer which appears in the outflow, h(t), is determined from the outflow concentration-time curve. Figure 2 shows the curves for a reference tracer (labeled albumin) that remains intravascular and for a tracer that leaves the capillary (⁸⁵Sr). The formula for the instantaneous extraction of the test tracer, E(t), is

$$E(t) = \frac{h_R(t) - h(t)}{h_R(t)}$$

where R indicates the intravascular reference tracer (here, albumin-⁵¹Cr), h_R(t) is the fraction of the intravascular tracer, and h(t) is the fraction of the test tracer (⁸⁵Sr or sucrose-¹⁴C) that appears in the outflow. In essence, it is possible to measure the fraction of the injected dose that is present in the venous outflow from the limb after one transcapillary passage. Although the venous blood includes blood from tissues other than bone, the blood from the other tissues merely dilutes the tracers and does not change their total amounts or

relative concentrations. Using the venous outflow from the limb maximizes the amount of blood collected and gives more accurate results than collecting nutrient-vein blood⁴.

The amount of test tracer extracted during a single passage through the capillary bed — that is, the extraction — is dependent on the permeability of the capillary, P , expressed as centimeters per second (cm s^{-1}); on the surface area available for exchange, S , in square centimeters per gram of bone tissue ($\text{cm}^2 \text{g}^{-1}$); and on the flow of the solute-carrying fluid in milliliters per 100 grams of bone per minute. In the case of ^{85}Sr , it is plasma that is the solute-carrying fluid, and its flow, F_s is measured in milliliters of plasma per minute per 100 grams of bone. Crone⁶ expressed this relationship as the permeability-surface area product, or PS:

$$PS = -F_s \log_e (1 - E)$$

The best measure of instantaneous extraction, $E(t)$, is the instantaneous extraction near the peak of the dilution curve, as illustrated in Figure 2. This peak extraction is called E_{max} . For these experiments, a reliable method of obtaining E_{max} was to choose the peak of the $E(t)$ curve that was nearest the peak of the reference curve — that is, nearest the peak of the dilution curve of the non-permanent reference tracer, which in these experiments was albumin- ^{51}Cr . The reason for this choice is that when the test-tracer curve crosses the curve of the intravascular reference tracer (Fig. 3) on the down slope, back-diffusion of the test tracers into the capillaries is occurring from the extravascular compartment, and this reverse flow decreases the estimate of extraction.

Previous studies of the pattern of blood flow in the region of a canine tibial fracture indicated that mineral deposition is directly related to blood flow¹⁶. In the present series of experiments, using alternative, more sensitive methods, we examined mechanisms of permeation of the tracer ^{85}Sr across the capillary membrane and attempted to establish more accurately the relationships between the extraction of the bone-seeking tracer ^{85}Sr , the blood flow, and the transcapillary passage of ^{85}Sr , a cation that is concentrated in bone. These techniques have been used in other organs such as the kidney, liver, lung, brain, and heart^{3,6}. Furthermore, if extraction changes little with increased flow, then determination of ^{85}Sr clearance provides a useful, inexpensive technique for the estimation of blood flow, especially in studies comparing bones of the right and left sides in the same dog.

Methods

Preparation of the Tibia

Using a standardized technique, the right tibia of adult male dogs was exposed and divided under anesthesia at the junction of the middle and distal thirds with an oscillating Stryker saw. The fracture was then displaced in a standard fashion, reduced, and fixed with a four-hole Vitallium plate applied to the medial surface with 1.9-centimeter screws. All dogs were able to bear weight without difficulty after three days.

At the time of the experiment, with the dog anesthetized, the nutrient artery of the right tibia was exposed by the transfibular route and cannulated with a PE 50 polyethylene tube, 0.58 millimeter in diameter, which was used for the indicator-dilution studies.

Blood Flow

When an indicator-dilution method is applied to the tibial nutrient artery, the extraction value obtained is for the entire capillary bed supplied by this artery. This bed is for the most part within the diaphyseal cortex and marrow¹⁴. In determining the permeability-surface

area product as described already, using the formula $PS = -F_s \log_e (1 - E)$, the relevant plasma flow, F_s , is the flow through this capillary bed since plasma carries the cation ^{85}Sr . If there are regional differences in perfusion, as is likely in a fractured tibia, error will be introduced by the use of a regional flow in the equation for permeability-surface area product. For example, the use of flow at the fracture site is likely to lead to an overestimation of permeability-surface area product for the whole tibia. A value for the mean tibial diaphyseal blood flow in these dogs was arrived at by using the iodoantipyrine washout technique¹² and recording washout curves from both the fracture site and the proximal part of the diaphysis. Estimations were made at two weeks (three dogs) and at twelve weeks (four dogs) after fracture. Two sodium iodide crystals of external gamma recorders were placed in contact with the skin, one over the fracture and the other over the proximal part of the diaphysis of the tibia, as previously described¹⁵. Control values were available from previous studies in this laboratory^{4,7}. An injection of fourteen microcuries of ^{125}I -labeled 4-iodoantipyrine (I-Ap) in one milliliter of saline solution was given into the nutrient artery, followed by one milliliter of saline solution to flush out the system. The fracture-site and proximal diaphyseal washout curves of ^{125}I -labeled 4-iodoantipyrine were recorded over a fifteen-minute period on a paper-strip analog meter (Fig. 4). The mean of these two flow values was used subsequently as an approximation of the total tibial diaphyseal blood flow. Plasma flows were determined on the basis of the large-vessel hematocrit for each dog:

$$F_s = (1 - \text{hematocrit}) F_{\text{I-Ap}} \text{ ml/100 g per min} \quad (1)$$

where F_s is plasma flow and $F_{\text{I-Ap}}$ in diaphyseal blood flow.

The tibial diaphyseal blood flow ($F_{\text{I-Ap}}$) in seven dogs was estimated as flow per 100 grams of bone per minute from the washout curve using a modification of the height/area method of Zierler (Fig. 4):

$$F_{\text{I-Ap}} = \frac{100 \lambda_b}{\rho_b} \cdot \frac{C^*(0) - C^*(T)}{\int_0^T C^*(t) dt} \quad (2)$$

where $F_{\text{I-Ap}}$ is blood flow, $C^*(0)$ is peak activity recorded over the tibia, $C^*(T)$ is activity recorded at fifteen minutes when recording was stopped, $C^*(t)$ is the count rate at time t , and λ_b/ρ_b is the ratio of the partition coefficient of whole bone to the specific gravity of whole bone, in the area counted by the external counter. The partition coefficient for whole bone is the counts per minute per milliliter of whole bone divided by the counts per minute per milliliter of blood.

Determination of the Ratio of the Partition Coefficient (λ) to the Specific Gravity (ρ) of Whole Bone ($\lambda_{\text{Whole Bone}}/\rho_{\text{Whole Bone}}$)

Blood flow per 100 grams of bone per minute in the proximal part of the diaphysis and in the fracture site was determined at two and twelve weeks after fracture in a separate series of experiments. For these calculations, weighted mean specific gravities of the tissues (mean based on the proportions of component tissue) making up the bone samples were used in Equation 2. Two dogs were used at each time period, and both tibiae in each dog were osteotomized and fixed with a plate and screws as previously described.

At the time of the experiment, each dog was anesthetized, the carotid artery and jugular vein were cannulated, and both renal arteries and veins were tied off to maintain high levels of circulating ^{125}I -labeled 4-iodoantipyrine¹⁷. At zero time, 500 microcuries of iodoantipyrine was injected into the jugular vein, and blood samples were obtained from the carotid artery

at ten, twenty, thirty, sixty, ninety, 120, 150, and 180 minutes. Based on previous work of Lopez-Curto and co-workers¹⁵ in this laboratory, who determined blood-bone equilibration curves for iodoantipyrine for the normal tibial diaphysis in the dog, we obtained the weighted mean partition coefficients of representative samples of the proximal part of the diaphysis (cortex and marrow) or of the fracture site (cortex and endosteal and periosteal callus). To do this, we removed the samples from one tibia at thirty minutes and from the other at three hours after injection of iodoantipyrine. The partition coefficient was calculated from the ratio of the blood and tissue samples obtained at thirty minutes from one tibia and at three hours from the other tibia. An oscillating saw was used to cut the samples, which were kept bloodless by first pouring liquid nitrogen over each sampling site. A Beckman counter was then used to count the isotopic activity in one milliliter of whole blood, one milliliter of plasma, and bone samples of known weight, one from the fracture site and the other from the proximal part of each of the tibiae studied. The mean counts in whole blood and in plasma were both used to determine the blood-bone and plasma-bone partition coefficients.

The blood-tissue partition coefficient, λ (counts per milliliter of tissue divided by counts per milliliter of blood), is a measure of the relative solubility of the test tracer in the tissue under study and in blood and is determined at blood-tissue equilibrium by the equation

$$\lambda_{\text{whole bone}} = \frac{\text{counts/min per ml tissue}}{\text{counts/min per ml blood}} \quad (3)$$

When more than one tissue is involved, as with cortex and marrow, a weighted mean partition coefficient is required. This is determined from the partition coefficients for each tissue by the equation

$$\begin{aligned} \lambda_{\text{whole bone}} &= [\lambda_c V_c + \lambda_m V_m] / [V_c + V_m] \\ &= [\lambda_c w_c / \rho_c + \lambda_m w_m / \rho_m] / [w_c / \rho_c + w_m / \rho_m] \end{aligned} \quad (4)$$

where λ_c is the blood-cortical bone partition coefficient, V_c is the volume of cortical bone in milliliters, w_c is the weight of cortical bone in grams, ρ_c is the specific gravity of cortical bone in grams per milliliters, and λ_m , V_m , w_m , and ρ_m , are the equivalent terms for marrow. The blood-cortical bone partition coefficient, λ_c , is 0.27 and the blood-marrow partition coefficient, λ_m , is 0.17, values derived from measurement of antipyrine space of bone¹⁵. The specific gravity of cortical bone is 1.94 grams and the specific gravity of marrow is taken as 0.94 gram per milliliter¹.

The specific gravities of the bone samples, $\rho_{\text{whole bone}}$, were determined by means of a chain gravitometer and the formula

$$\text{specific gravity} = \frac{\text{weight in air}}{\text{weight in air} - \text{weight in water}} \quad (5)$$

Technical difficulties prevented the use of this method when marrow was present in the proximal part of the diaphysis. In this situation, a weighted mean specific gravity was determined by using the equation

$$\rho_{\text{whole bone}} = [w_c + w_m] / [w_c / \rho_c + w_m / \rho_m] \quad (6)$$

and the known ratio by weight of cortex to marrow in the tibiae of dogs, which is 9: 1⁴.

The solubility of iodoantipyrine in erythrocytes is more than would be expected from their water content, the red cell-plasma partition coefficient being 1.1 as reported by Effros and

Chinard. Therefore, to determine the plasma-tissue partition coefficient, whether the tissue is marrow or cortical, periosteal, or endosteal bone, it is necessary to correct the counts per minute per milliliter of blood in Equation 3, which reflects the blood within the bone — that is, the blood-bone (bb). Therefore, we calculated the blood-bone partition coefficient, λ_{bb} , for iodoantipyrine in bloods with varying hematocrits by using the plasma-bone partition coefficient, λ_{pb} , and the equation

$$\lambda_{bb} = \lambda_{pb} [1.1 \times 0.65 \times \text{hematocrit} + 0.94 (1 - \text{hematocrit})] \quad (7)$$

where 0.65 is the water content of red cells and 0.94 is the water content of plasma.

Extraction of Mineral

This was determined in twenty-nine dogs: nineteen with and ten without fracture, the ten without fracture serving as controls. A one-milliliter mixture containing five microcuries of ^{85}Sr , five microcuries of sucrose- ^{14}C , and fifteen microcuries of albumin- ^{51}Cr was infused into the nutrient artery of the twenty-nine dogs by means of a Harvard pump during forty-seven seconds. ^{85}Sr was used as a calcium tracer because of its long half-life (185 days compared with 4.6 days for ^{47}Ca). Work in this laboratory by one of us (G. J. L.) indicated that the extractions of ^{85}Sr and of ^{47}Ca are identical. Sucrose, in contrast to strontium, is a diffusible inert substance that penetrates the capillary walls but not the cell walls. Therefore, it fills only the extravascular space. Chromium-labeled albumin was used as the intravascular reference tracer. The whole of the venous outflow from the ipsilateral femoral vein, F_v , was collected in ten-second samples for three minutes, a total of eighteen samples. Aliquots of 0.3 milliliter of plasma from each ten-second sample were then put into ten milliliters of Insta-Gel (Packard) and counted in a Nuclear Chicago Mark II liquid scintillation system. The three radioisotopes were separated by the technique described by Davies and co-workers, the data were analyzed on a Control Data 3500 computer, and indicator-dilution curves were constructed from the results (Figs. 5-A and 5-B).

The extraction was determined from indicator-dilution curves like the ones shown in Figure 2. The fractions of the injected reference tracer, $h_R(t)$, and test tracer, $h(t)$, appearing in the venous outflow per second, were calculated by using the equation

$$h(t) = \frac{F_v}{I} \cdot C(t) \quad (8)$$

In this relationship, $h(t)$ or $h_R(t)$ is the fraction of the injected dose of the test or reference tracer per second appearing in the outflow from the femoral vein, F_v , in milliliters per second; I is the total injected dose expressed as counts per minute; and $C(t)$ is the concentration of tracer in the femoral venous blood in counts per minute at time t . If the concentration of albumin (the reference tracer) is zero in the femoral vein at three minutes, it can be assumed that all of the albumin is out of the bone and that the area under the curve for the reference tracer is unity. Hence

$$h(t) = \frac{F_v C(t)/I}{\int_0^3 h_R(t) dt} \quad (9)$$

The instantaneous extraction $E(t)$ can then be calculated from the formula

$$E(t) = \frac{h_R(t) - h(t)}{h_R(t)} \quad (10)$$

The maximum instantaneous extraction, $E_{\max}$, was taken to be the best indicator of unidirectional transcappillary extraction before back-diffusion became appreciable³.

Permeability

Given the maximum extraction ($E_{\max}$) and the plasma flow (F_s), the permeability-surface area product (PS) of the capillaries can be determined, and with this it is possible to derive information about the permeability of the capillary walls from the formula developed by Renkin and by Crone⁶:

$$PS = -F_s \log_e (1 - E_{\max}) \quad (11)$$

In the present experiment, in which three substances were injected concurrently, including the reference tracer albumin-⁵¹Cr, the test tracer strontium (⁸⁵Sr), and the freely diffusible tracer sucrose-¹⁴C, we used the technique described by Davies and associates, who applied it to the study of the extraction of strontium and the capillary permeabilities of strontium and sucrose in bone. If the extractions of two test substances, a and b, are determined simultaneously, then the capillary surface area (s) and plasma flow (F_s), which are unknown, must be the same for each test tracer. Hence, a permeability ratio (P_a/P_b) may be determined⁵ from Equation 11 for the two substances, as follows:

$$\frac{P_a/S}{P_b/S} = \frac{-F_s \log_e (1 - E_{\max_a})}{-F_s \log_e (1 - E_{\max_b})} \quad (12)$$

and in this ratio the surface area and flow cancel out as indicated, since they are the same for each isotope.

The permeability-surface area product can be calculated because $E_{\max}$ for each substance is known, and the plasma flow (F_s) can be calculated from the tibial diaphyseal blood flow (F_{1-Ap}) as determined from Equation 2 and the equation

$$F_s = (1 - \text{hematocrit}) F_{1-Ap} \text{ ml/100 g per min} \quad (13)$$

Net Extraction

The net extraction (E_{net}), an area-weighted mean extraction, is equal to the area between the two indicator-dilution curves up to time t, which was three minutes in this experiment, divided by the area under the reference curve up to this time (Fig. 3). The net extraction is used to calculate flow from clearance:

$$E_{\text{net}} = \frac{\int_0^T [h_R(t) - h(t)] dt}{\int_0^T [h_R(t)] dt} \quad (14)$$

Mean Transit Time of Albumin ($\bar{t}_{\text{alb}}$)

The mean transit time of a reference tracer may be used as an indication of the ability of the vascular bed to respond to a known increase in flow. Since the reference tracer albumin-⁵¹Cr does not leave the vascular space during the time of the experiment, it is possible to determine the mean time for the transit of albumin from the point of entry into the nutrient artery to the point of exit from the femoral vein, $\bar{t}_{\text{alb}}$, by using the outflow dilution curve.

The mean transit time, $\bar{t}$, may be calculated from the equation for $\bar{t}'_{alb}$, the sum of the mean time for injection and the mean transit time for the tracer to move from the nutrient artery to the femoral vein:

$$\bar{t}'_{alb} = \frac{\sum_{i=1}^{i=n} C_i t_i}{\sum_{i=1}^{i=n} C_i} \quad (15)$$

where C_i is the concentration of albumin at the i^{th} time of sampling, t_i , expressed as a fraction of injectate of albumin. The effects of recirculation are eliminated by plotting the curve on a logarithmic scale and extrapolating the exponential portion of the downslope of the albumin concentration-time curve until the concentration of albumin is 1 per cent of the peak concentration. These new values for $C(t)$ are then used in the calculation of $\bar{t}'_{alb}$, which includes the time for the injection (forty-seven seconds) and therefore is equivalent to the sum of the mean entry time of the tracer into the system and the mean transit time for the tracer to go from the nutrient artery to the femoral vein. Therefore, the true transit time of albumin, $\bar{t}_{alb}$, is obtained by subtracting the time for injection, t_{in} , which is $1/2 (t_{end} - t_{start})$, from $\bar{t}'_{alb}$

$$\bar{t}_{alb} = \bar{t}'_{alb} - 1/2 (t_{end} - t_{start}) \quad (16)$$

where t_{end} is the time when the constant-rate infusion of the tracer was stopped, and t_{start} is the time when the injection of the tracer was started, ordinarily at time zero. Therefore, $t_{in} = 1/2 (47 \text{ sec} - \text{zero})$ or 23.5 seconds (Figs. 5-A and 5-B). A more detailed analysis was published by Bassingthwaighe².

Results

Tibial Blood Flow

Using Equation 2 and the values for the ratio of the partition coefficient for whole bone to the specific gravity of whole bone ($\lambda_{\text{whole bone}} / \rho_{\text{whole bone}}$) at two weeks and twelve weeks after fracture (Table I), the mean fracture-site blood flow, F_{I-AP} , was found to be 6.65 ± 0.45 milliliters per 100 grams of bone per minute at two weeks and 4.77 ± 0.95 at twelve weeks. The mean proximal diaphyseal flow was 4.42 ± 2.94 milliliters per 100 grams of bone per minute at two weeks and 3.15 ± 0.53 at twelve weeks (Table II). The mean tibial plasma flow, F_s , calculated from these measurements, was 2.93 ± 1.09 milliliters per 100 grams of bone per minute at two weeks and 2.05 ± 0.38 at twelve weeks.

Of the four dogs that were studied at twelve weeks, two showed roentgenographic evidence of union and the other two showed incomplete union.

Extraction

Typical outflow-dilution curves following a forty-seven-second infusion of the three tracers into the nutrient artery two weeks after fracture are shown in Figure 5-A, and the corresponding extractions, calculated by means of Equation 10, are shown in Figure 5-B. The mean peak extractions (E_{max}) for strontium two weeks after fracture were 0.76 ± 0.14 of the amount injected in the nine dogs with fractured tibiae and 0.77 ± 0.09 of the amount in the ten dogs with intact tibiae. Similarly, at two weeks the mean peak extractions for sucrose were 0.50 ± 0.13 in the nine dogs with fractures and 0.56 ± 0.13 in the ten dogs with intact tibiae (Table III). However, there was no statistically significant difference between the mean peak extraction of strontium and that of sucrose, even though the bone blood flow at

the fracture site at two weeks was increased from a control value of 1.5 to a value of 6.65 milliliters per 100 grams of bone per minute (Fig. 6).

The mean net extractions of ^{85}Sr (E_{net}) at two weeks, as calculated with Equation 14 and shown in Figure 5-B, were 0.70 ± 0.15 in the nine dogs with fractured tibiae and 0.62 ± 0.14 in the ten dogs with unfractured tibiae. The mean net extractions of sucrose at two weeks, on the other hand, were 0.29 ± 0.16 for the fractured tibiae (nine dogs) and 0.27 ± 0.12 for the unfractured tibiae (ten dogs). However, there were no statistically significant differences between the net extractions at two and twelve weeks except that at twelve weeks the net extraction of sucrose in the fractured tibiae was increased (Table III). This high E_{net} was probably the result of the larger volume in which the sucrose was distributed. The extravascular volume at the fracture site was increased because of the less-than-complete mineralization of callus, indicated by the low specific gravity of the tibia at this time. The values for dogs studied at six weeks were similar to those for the dogs studied at two weeks.

Permeability-Surface Area Products

Values for the permeability-surface area products of the capillary beds in the fractured tibiae were calculated by using Equation 11. As shown in Table IV, the mean permeability-surface area products for strontium were 5.19 ± 1.53 milliliters per 100 grams of bone per minute at two weeks and 3.24 ± 1.01 milliliters per 100 grams of bone per minute at twelve weeks. Values in an unfractured tibia, as determined by Cofield and associates, were 0.63 ± 0.29 milliliter per 100 grams of bone per minute and as determined in this laboratory by Maltby (unpublished data), 0.64 ± 0.32 . Therefore the permeability-surface area product is influenced by blood flow, as is seen in Figure 7, and as the flow increases, so does the permeability-surface area product.

The permeability ratio (the permeability for ^{85}Sr to that for sucrose- ^{14}C) was calculated by using Equation 12, and the results are shown in Table IV. At two weeks, the mean increase in the ratio was from the mean control value of 1.88 ± 0.17 to a value of 2.05 ± 0.62 , but subsequently the ratio returned to 1.44 ± 0.24 at twelve weeks.

As bone blood flow increased, the permeability-surface area products increased. This change is probably accounted for by an increase in the surface area, as more of the capillary bed opens up at the higher flow rates.

Transit Time

The mean transit time of albumin from the nutrient artery to the femoral vein was calculated by using Equation 15. There was no significant change in the mean transit time of the intravascular tracer albumin for up to twelve weeks after fracture. The control mean time was 45 ± 10 seconds, and the other times were 40 ± 9 seconds at two weeks, 48 ± 7 seconds at six weeks, and 40 ± 8 seconds at twelve weeks.

Discussion

In muscle, electrolyte and lipid-insoluble substances are believed to diffuse through the capillary walls either through so-called pores composed of chains of intracellular vesicles or through clefts that form channels between the endothelial cells. The lipid-soluble substances, on the other hand, are known to diffuse freely through the endothelial cells that compose the capillary, as well as through the pores and clefts^{13,19}. Studies in this laboratory indicated that the walls of the capillaries in bone function similarly¹¹. Furthermore, studies of normal bone indicated that free diffusion is the principal mechanism by which ions and solutes move into bone⁷.

Stated in another way, the movement of ions or molecules through the capillary walls into the extravascular space, throughout which they are distributed, is dependent to some extent on the size of these ions or molecules, on whether they are hydrophilic or lipophilic, and on the volume of the extravascular space. Therefore, the extraction of the smaller Sr^{++} ion will be greater than the extraction of the larger sucrose molecule since many more Sr^{++} ions than sucrose molecules can be accommodated in any given space. In addition, bone-seeking ions such as Sr^{++} and Ca^{++} can move into the mineral phase of bone as well as into the interstitial fluid space. In contrast to sucrose and Sr^{++} , the lipophilic iodoantipyrine diffuses through the endothelial cells as well as through the pores and clefts between these cells, and its diffusion is unrestricted and completely free.

Using mixtures of radionuclides composed of a reference tracer such as labeled albumin (albumin- ^{51}Cr), a permeable tracer ion such as ^{85}Sr , ^{42}K , or ^{47}Ca , and a tracer molecule such as sucrose- ^{14}C , antipyrine- ^{14}C , or penicillin- ^{14}C , and Equation 12, it is possible to determine a permeability ratio — the ratio of the permeability of one tracer substance to the permeability of another — because the diffusion coefficients of these ions or solutes are known, as are their hydrophilic or lipophilic properties. Therefore, by simultaneously injecting labeled sucrose and ^{85}Sr , we were able to compare the permeability ratio of the two substances at two weeks with the ratio at twelve weeks after fracture, and to determine that the two ratios were very similar, even though the product of the capillary permeability and surface area increased during the ten weeks. The most logical explanation for this similarity is that the permeability is relatively unchanged, whereas the surface area increases. To state this another way, the capillary bed of the fractured bone is increased by recruitment, more unopened capillaries becoming perfused, as observed in isometrically contracting dog hearts⁸. Additional evidence supporting this mechanism is the similarity of the mean transit times of albumin from the nutrient artery of the tibia to the femoral vein in the control and in the fractured limbs. We also found that the net extraction, E_{net} , of ^{85}Sr did not change with increases in blood flow.

It is our conclusion that the initial response in bone to an increase in blood flow is an increase in surface area of the vascular bed which provides a greater area for mineral exchange.

We also conclude that because the extraction of the tracer, ^{85}Sr , does not change with increases in flow, the described technique using clearance is a useful and inexpensive method to define bone blood flow, particularly when comparing fractured bones in the same dog.

Acknowledgments

The authors thank Mr. Glenn Christensen for his invaluable help.

References

1. Allen TH, Krzywicki HJ, Roberts JE. Density, Fat, Water and Solids in Freshly Isolated Tissues. *J Appl Physiol.* 1959; 14:1005–1008. [PubMed: 13792786]
2. Bassingthwaighe, JB. The Measurement of Blood Flows and Volumes by Indicator Dilution. In: Ray, CD., editor. *Medical Engineering*. Vol. Chapter 20. Chicago: Year Book Medical Publishers; 1973. p. 246-260.
3. Bassingthwaighe JB. A Concurrent Flow Model for Extraction during Transcapillary Passage. *Circ Res.* 1974; 35:483–503. [PubMed: 4608628]
4. Cofield RH, Bassingthwaighe JB, Kelly PJ. Strontium-85 Extraction During Transcapillary Passage in Tibial Bone. *J Appl Physiol.* 1975; 39:596–602. [PubMed: 1104562]

5. Crone, Christian. Does "Restricted Diffusion" Occur in Muscle Capillaries? *Proc Soc Exper Biol and Med.* 1963; 112:453–455. [PubMed: 14041407]
6. Crone, Christian. The Permeability of Capillaries in Various Organs as Determined by Use of the 'Indicator Diffusion' Method. *Acta Physiol Scandinavica.* 1963; 58:292–305.
7. Davies DR, Bassingthwaighe JB, Kelly PJ. Transcapillary Exchange of Strontium and Sucrose in Canine Tibia. *J Appl Physiol.* 1976; 40:17–22. [PubMed: 1248978]
8. Durán WN, Marsicano TH, Anderson RW. Capillary Reserve in Isometrically Contracting Dog Hearts. *Am J Physiol.* 1977; 233:H276–H281. [PubMed: 329689]
9. Effros RM, Chinard FP. The In Vivo pH of the Extravascular Space of the Lung. *J Clin Invest.* 1969; 48:1983–1996. [PubMed: 4898722]
10. Hughes SPF, Davies DR, Bassingthwaighe JB, Knox FG, Kelly PJ. Bone Extraction and Blood Clearance of Diphosphonate in the Dog. *Am J Physiol.* 1977; 232:H341–H347. [PubMed: 402827]
11. Kelly PJ, Bassingthwaighe JB. Studies on Bone Ion Exchanges Using Multiple-Tracer Indicator-Dilution Techniques. *Fed Proc.* 1977; 36:2634–2639. [PubMed: 913623]
12. Kelly PJ, Yipintsoi Tada, Bassingthwaighe JB. Blood Flow in Canine Tibial Diaphysis Estimated by Iodoantipyrine-¹²⁵I Washout. *J Appl Physiol.* 1971; 31:38–47. [PubMed: 5556961]
13. Landis, EM.; Pappenheimer, JR. Exchange of Substances Through the Capillary Walls. In: Hamilton, WF.; Dow, P., editors. *Handbook of Physiology, Section 2, Circulation.* Vol. 2. Washington, D.C: American Physiological Society; 1963. p. 961-1034.
14. Lopez-Curto JA, Bassingthwaighe JB, Kelly PJ. Microvascular Relationships of Cortex and Marrow of the Canine Tibia. *Trans Orthop Res Soc.* 1977; 2:160.
15. Lopez-Curto JA, Hughes SPF, Kelly PJ, Bassingthwaighe JB. Interstitial Fluid Space in Bone. *Fed Proc.* 1976; 35:850. abstract.
16. Paradis GR, Kelly PJ. Blood Flow and Mineral Deposition in Canine Tibial Fractures. *J Bone and Joint Surg.* Mar.1975 57-A:220–226. [PubMed: 1112848]
17. Polimeni PI. Extracellular Space and Ionic Distribution in Rat Ventricle. *Am J Physiol.* 1974; 227:676–683. [PubMed: 4413398]
18. Renkin EM. Transport of Potassium-42 from Blood to Tissue in Isolated Mammalian Skeletal Muscles. *Am J Physiol.* 1959; 197:1205–1210. [PubMed: 14437359]
19. Simionescu, Nicolae; Simionescu, Maia; Palade, GE. Permeability of Muscle Capillaries to Exogenous Myoglobin. *J Cell Biol.* 1973; 57:424–452. [PubMed: 4696549]
20. Zierler KL. Equations for Measuring Blood Flow by External Monitoring of Radioisotopes. *Circ Res.* 1965; 16:309–321. [PubMed: 14270567]

Fig. 1.
Tracers are injected into the nutrient artery and blood is collected from the femoral vein.

Fig. 2.

Top: Indicator-dilution curves showing changes in the fraction of the reference tracer, $h_R(t)$, and in the fraction of the test tracer, $h(t)$, in the outflow in the femoral vein with time.

Bottom: Instantaneous extraction, $E(t)$, and the maximum instantaneous extraction, E_{max} , calculated from the indicator-dilution curves by the equation shown (see text).

Fig.3.

Net extraction, E_{net} , is equivalent to the shaded areas between the indicator-dilution curves of the reference and of the test tracers divided by the total area beneath the curve of the reference tracer up to time t (see Fig. 2). The cross-hatched area to the right of where the curves cross indicates the extent to which back-diffusion has become greater than efflux from the capillaries. The net extraction, E_{net} , shown in the bottom graph, is calculated by using Equation 14, as shown (see text).

Fig. 4.

Proximal tibial diaphyseal blood flow measured from the washout curve determined by a modification of the height/area method of Zierler (Equation 2). $C^*(0)$ is peak activity recorded over the tibia, $C^*(T)$ is activity recorded at fifteen minutes, when recording was stopped, and $C^*(t)$ is the counts per minute at time t (see text).

Fig. 5.
A Indicator-dilution curves for albumin- ^{51}Cr , sucrose- ^{14}C , and ^{85}Sr , after a forty-seven-second infusion into the nutrient artery of a dog's tibia.

B Corresponding instantaneous extractions, $E(t)$, calculated by Equations 10 and 14. Maximum instantaneous extraction, $E_{\max}$, is the highest initial instantaneous extraction, which in this case is at thirty seconds and not eighty-six seconds, when back-diffusion has occurred (see Fig. 3). Net extraction, $E_{\text{net}}(t)$, is 1.0 minus the ratio of the fraction of the injected tracer, ^{85}Sr , that has emerged at time t divided by the fraction of the non-permeant albumin which has emerged by that time; therefore, in the late phase, such as at 180 seconds. $E_{\text{net}}(t) = 180$ is a measure of the retention of strontium by the bone at this time. Mean transit time of injection, T_{in} , for albumin is 23.5 seconds.

Fig. 6. Plots of the peak instantaneous extractions, $E_{\max}$, and of net extractions, E_{net} , of strontium and sucrose against the plasma flow including least-squares regression lines. Note that peak extraction ($E_{\max}$), especially of strontium, is not affected by the amount of plasma flow.

Fig.7.
Plot of permeability-surface area product, PS, against flow of plasma, F_s, to demonstrate linear relationship.

TABLE I

Values for the Ratio of the Blood-Bone Partition Coefficient for the Whole Bone ($\lambda_{\text{whole bone}}$) to the Specific Gravity of the Whole Bone ($\rho_{\text{whole bone}}$) in Four Dogs

		Fracture Site			Proximal Cortex			Proximal Diaphysis		
		λ	ρ	λ/ρ	λ	ρ	λ/ρ	λ	ρ	λ/ρ
Dog 8. 2 wks.	30 mins.	0.59	1.39	0.42	0.49	1.48	0.35	0.44	1.39	0.32
	180 mins.	0.51	1.52	0.34	0.63	1.82	0.35	0.55	1.67	0.33
Dog 9. 2 wks.	30 mins.	0.54	1.69	0.32	0.32	1.79	0.20	0.30	1.64	0.18
	180 mins.	0.53	1.64	0.32	0.64	1.61	0.40	0.57	1.49	0.38
Mean $\pm$ S. D.		0.54 $\pm$ 0.03	1.56 $\pm$ 0.13	0.35 $\pm$ 0.05	0.52 $\pm$ 0.15	1.68 $\pm$ 0.16	0.33 $\pm$ 0.09	0.47 $\pm$ 0.12	1.55 $\pm$ 0.13	0.30 $\pm$ 0.09
S.E.M.		0.02	0.07	0.02	0.07	0.08	0.04	0.06	0.07	0.04
Dog 10. 12 wks.	30 mins.	0.81	1.32	0.61	*			0.52	1.48	0.35
	180 mins.	0.71	1.84	0.39	0.51	1.79	0.28	0.46	1.64	0.28
Dog 11. 12 wks.	30 mins	0.64	1.35	0.47	0.49	1.51	0.32	0.44	1.41	0.31
	180mins.	0.85	1.34	0.63	0.56	1.70	0.33	0.50	1.56	0.32
Mean $\pm$ S.D.		0.75 $\pm$ 0.10	1.46 $\pm$ 0.25	0.53 $\pm$ 0.11	0.52 $\pm$ 0.04	1.67 $\pm$ 0.14	0.31 $\pm$ 0.03	0.48 $\pm$ 0.04	1.52 $\pm$ 0.10	0.32 $\pm$ 0.03
S.E.M.		0.05	0.13	0.03	0.02	0.08	0.02	0.02	0.05	0.01

* In this bone the marrow cavity was filled with endosteal callus, so the proximal diaphysis was sampled in one piece.

Blood Flow at Fracture Site($F_{I,AP}$ Milliliters per 100 Grams of Bone per Minute) and Mean Tibial Plasma Flow(F_s , Milliliters per 100 Grams of Bone per Minute)

TABLE II

	Fracture Site		Proximal Diaphysis		Mean Tibial Plasma	
	$F_{I,AP}$	F_s	$F_{I,AP}$	F_s	F_s	F_s
Two weeks						
Dog 1	6.35	3.05	4.31	2.07	2.56	2.56
Dog 2	7.16	4.08	7.42	4.23	4.16	4.16
Dog 3	6.43	3.34	1.54	0.80	2.07	2.07
Mean $\pm$ S.D.	$6.65 \pm 0.45^*$	3.49 ± 0.53	4.42 ± 2.94	2.37 ± 1.73	2.93 ± 1.09	2.93 ± 1.09
S.E.M.	0.26	0.18	1.70	1.00	0.63	0.63
Twelve weeks						
Dog 4	5.80	2.96	3.60	1.84	2.40	2.40
Dog 5	3.54	1.80	2.42	1.23	1.52	1.52
Dog 6	4.65	2.47	3.08	1.63	2.05	2.05
Dog 7	5.08	2.64	3.48	1.81	2.23	2.23
Mean $\pm$ S.D.	$4.77 \pm 0.9^{\dagger}$	2.47 ± 0.49	3.15 ± 0.53	1.63 ± 0.28	2.05 ± 0.38	2.05 ± 0.38
S.E.M.	0.47	0.24	0.27	0.14	0.19	0.19

* Mean flow ($F_{I,AP}$) at fracture site at two weeks, which was 6.65 ± 0.45 , is different from that at twelve weeks, which was 4.77 ± 0.95 ($p < 0.02$).

† Comparison of mean flow at fracture site, 4.77 ± 0.95 , versus that in the proximal part of the tibia, 3.15 ± 0.53 , shows no significant difference ($p = 0.20$).

TABLE III

Extraction of Strontium and Sucrose in Twenty-nine Dogs (Mean + S.D.)

	Control N = 10	Fractured Tibiae		
		2 Weeks N = 9	6 Weeks N = 5	12 Weeks N = 5
⁸⁵ Sr				
E _{max}	0.77 ± 0.09	0.76 ± 0.14	0.68 ± 0.18	0.74 ± 0.10
E _{net} (3 mins.)	0.62 ± 0.14	0.70 ± 0.15	0.55 ± 0.13	0.68 ± 0.13
Sucrose- ¹⁴ C				
E _{max}	0.56 ± 0.10	0.50 ± 0.13	0.53 ± 0.20	0.63 ± 0.10
E _{net} (3 mins.)	0.27 ± 0.12	0.29 ± 0.16	0.31 ± 0.07	0.52 * ± 0.12

* Different from control value p < 0.002.

TABLE IVPermeability-Surface Area Product and Permeability Ratio (Mean $\pm$ S.D.)^{*}

Study Group	Permeability-Surface Area Product (<i>ml/100g/min</i>)	Permeability Ratio
2 weeks	5.19 $\pm$ 1.53	2.05 $\pm$ 0.62
12 weeks	3.24 $\pm$ 1.01	1.44 $\pm$ 0.24
Control [†]	0.78	1.88 $\pm$ 0.17
	0.63 $\pm$ 0.29 (S.E.M.)	2.10 $\pm$ 0.4 (S.E.M.)
	N = 12 ⁴	N = 14 ⁷

^{*}See text.[†]These data are taken from previous work.