Extraction Ratio and Bone Clearance of Sr\textsuperscript{85} as a Measure of Effective Bone Blood Flow

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Blood flow through bone is important functionally\textsuperscript{1} but its quantitative measurement is remarkably difficult because of the involved vascular pattern and the rigidity of the tissue. Post and Shoemaker\textsuperscript{2} have devised an ingenious technique for collecting the venous outflow from the ends of the femur, and in 139 determinations in normal unanesthetized dogs, observed an average blood flow of 11 ml per minute (range 4.3-25.1 ml per minute). While the results are of great interest, their technique involves delicate and complex surgical procedures, and is not readily adaptable to studies on other bones or in small animals. An indirect and more generally applicable method, based on initial bone clearance of Ca\textsuperscript{44}, has been proposed by Frederickson et al.\textsuperscript{8} This procedure uses the same principle as that employed in determining effective renal plasma flow by diodrast\textsuperscript{4} or PAH\textsuperscript{5} clearance. The latter is defined as the volume of blood or plasma "cleared" of the substance per minute, and if clearance is complete (i.e., the concentration in the venous outflow from the organ is essentially zero) this will be equivalent to the actual rate of blood flow. The efficiency of removal of the substance by the organ is expressed by the extraction ratio \( ER = (A-V)/A \), and A and V are the concentrations of the substance in arterial and venous blood respectively. The kidney is highly efficient in removing diodrast and PAH from blood, with an \( ER \) of approximately 0.90, so that the clearance of these substances gives a useful quantitative measure of effective renal plasma flow.

Frederickson et al.\textsuperscript{3} suggested that Ca\textsuperscript{44} should also be removed with great efficiency from the blood flowing through bone, by exchange with the very large reservoir of non-radioactive calcium that is present. Weinman et al.\textsuperscript{6} studied skeletal blood flow in dogs by this method, using Ca\textsuperscript{45} and Sr\textsuperscript{85}. They found that the clearances of both these substances were essentially the same. The blood concentration of Sr\textsuperscript{85} changes rapidly during the clearance period, and this must be considered in the calculations. The validity of Sr\textsuperscript{85} clearance as a measure of bone blood flow depends on the consistency and completeness of removal of the isotope by bone during a single passage. This can be evaluated from the Sr\textsuperscript{85} extraction ratio.

The present paper describes a method for estimating this ratio using Sr\textsuperscript{85} and a non-diffusible dye, Evans blue (T-1824). Both were injected simultaneously into the nutrient artery of the tibia. Blood was collected from the femoral vein during the next five minutes and the percentage of the injected dose of each substance in this blood was determined. The extraction ratio for Sr\textsuperscript{85} was calculated from these data. It appeared to be sufficiently high and consistent to justify the use of initial bone clearance of Sr\textsuperscript{85} as a measure of effective bone blood flow. On this basis, rates of blood flow were then determined for a number of typical bones in dogs and rabbits.

Methods

A. EXTRACTION RATIO

The experiments were done on 10 adult mongrel dogs (weight 18 to 25 kg) which had been fasted overnight and anesthetized with pentobarbital (30 mg/kg) iv. In the dog, in contrast to man, the nutrient artery to the tibia arises normally from the anterior tibial artery rather
than from the posterior tibial artery. To expose the nutrient artery a longitudinal incision was made along the medial border of the fibula from a point two inches proximal to its head to the junction of the middle and lower third of the leg. After the superficial fascia was incised, the peroneal muscles were retracted laterally and the extensor muscles were displaced medially. The anterior tibial artery and vein, together with the deep peroneal nerve, were exposed anterior to the interosseous membrane. The artery was dissected free and its branches to muscle in the upper portion were ligated. In most dogs the nutrient artery originates from the anterior tibial artery within the first two to three inches distal to the point where it passes through the interosseous membrane and is directed downward and posteriorly to enter the nutrient foramen. In four dogs, the nutrient artery arose from the anterior tibial artery before it penetrated the membrane, and in these animals the approach was made through the popliteal space. The vessels were handled with great care to avoid injury or spasm.

The femoral vein on the same side was exposed in the femoral triangle and prepared for cannulation. The animal was then given 1 mg heparin/kg iv, and the vein was cannulated. The control blood was collected from this vein for several minutes. The anterior tibial artery was then ligated immediately distal to the origin of the nutrient artery and 1 ml of a solution containing 5 μc Sr⁸⁵ and 3.75 mg Evans blue (T-1824) was injected slowly (during one minute) into the nutrient artery at its origin, using a no. 23 gauge needle. The blood flow through the artery during this period is estimated to be between 4 and 8 ml. Immediately after the start of this injection, femoral vein blood was collected for five minutes in the first group (five dogs), and at one-minute intervals for five minutes in the second group. The percentage of the injected dose of Sr⁸⁵ in the collected blood was determined using a scintillation detector. The blood was centrifuged and the percentage of the injected dose of dye in plasma was determined with a Klett-Summerson photoelectric colorimeter using a green filter. This measured the fraction of the tagged blood that, after entering the nutrient artery, was ultimately recovered from the femoral vein. The general procedure is illustrated in figure 1.

At the end of the five-minute collection period, immediate cardiac arrest was produced by injecting 10 ml 6% EDTA (ethylene diaminetetraacetate) into the left ventricle and the tibia was removed speedily for Sr⁸⁵ analysis. The bone was cleaned, dried, and ashed at 600°C for 24 hours and then dissolved in 1 normal HNO₃. Sr⁸⁵ was determined on an aliquot of this sample, and the total uptake by tibia was expressed as a percentage of the administered dose.

B. DETERMINATION OF INITIAL Sr⁸⁵ CLEARANCE BY BONE

Dogs were fasted overnight and anesthetized with pentobarbital as described above. The carotid artery was cannulated on one side for rapid withdrawal of arterial samples, while the jugular vein on the opposite side was exposed. A dose of 5 to 10 μc/kg of carrier-free Sr⁸⁵ in 1 ml physiological saline was injected rapidly into the vein (in less than one second), and blood samples were withdrawn from the carotid artery at ten seconds and one, two, three, four, and five minutes following this injection. The Sr⁸⁵ in 1 ml aliquots of these blood samples was determined using a two-inch NaI scintillation detector and was compared with...
MEASUREMENT OF EFFECTIVE BONE BLOOD FLOW

FIGURE 2

Activity (% injected dose of Sr\textsuperscript{85} per ml) in whole blood for the first five minutes after rapid injection into the jugular vein of a dog.

At the end of the five-minute period following injection of Sr\textsuperscript{85}, immediate cardiac arrest was produced by intracardiac injection of 10 to 15 ml 6% disodium EDTA. The femur, tibia, humerus, and talus were quickly removed from the body, cleaned and weighed. The bones were then dried and ashed as described above. This ash was dissolved in 1 N HNO\textsubscript{3} and made up to an appropriate volume (50 to 250 ml) using distilled water. The Sr\textsuperscript{85} present in a 1 ml aliquot was determined with a scintillation detector and compared with a 1 ml aliquot of the standard.

Calculations

Symbols: % dose Eb = percentage of the dose of Evans blue injected into the nutrient artery.
% dose Sr\textsuperscript{85} = percentage of the dose of Sr\textsuperscript{85} injected into the nutrient artery.
(% dose Eb\textsubscript{f v}) = % Eb collected from femoral vein.
(% dose Sr\textsuperscript{85}\textsubscript{f v}) = % Sr\textsuperscript{85} collected from femoral vein.
Ut = % Sr\textsuperscript{85} taken up by the tibia in t minutes.
S = concentration of Sr\textsuperscript{85} in blood expressed as % Sr\textsuperscript{85}/ml.
ER = extraction ratio for Sr\textsuperscript{85} = \((A-V)/A\) = \((Q_A-Q_V)/Q_A\)

where A and V are the arterial and venous concentrations of Sr\textsuperscript{85} in blood entering and leaving bone, and \(Q_A\) and \(Q_V\) represent the quantity of Sr\textsuperscript{85} as % dose Sr\textsuperscript{85} in the blood entering and leaving bone during the clearance period.

Extraction Ratio

If all the blood containing Evans blue and Sr\textsuperscript{85} which entered the nutrient artery was subsequently collected from the femoral vein, \((%\ dose\ Eb\textsubscript{f v})\) should equal 100%, \(Q_A\) would be 100% and \(Q_V\) would equal \((%\ dose\ Sr\textsuperscript{85}\textsubscript{f v})\). The extraction ratio would be given by the formula:

\[
ER = \frac{100\% - (%\ dose\ Sr\textsuperscript{85}\textsubscript{f v})}{100\%}
\] (1)

However, less than 100% of the dose of Evans blue was in fact collected from the femoral vein. The small difference (13%) probably represents blood which has bypassed the femoral vein and has returned by other venous channels. The labelled blood entering the nutrient artery which was ultimately collected from the femoral vein should be reduced by this amount, and the proportion would be represented by \((%\ dose\ Eb\textsubscript{f v})\). In this case, \(Q_A\) = \((%\ dose\ Eb\textsubscript{f v})\) and the equation for extraction ratio becomes:

\[
ER = \frac{(\%\ dose\ Eb\textsubscript{f v}) - (\%\ dose\ Sr\textsuperscript{85}\textsubscript{f v})}{(\%\ dose\ Eb\textsubscript{f v})}
\] (2)

This equation has been used in calculating the data presented in this paper.

Recovery of Sr\textsuperscript{85}

The "recovery" of the injected dose of Sr\textsuperscript{85} was calculated on the basis of the five-minute uptake by the tibia plus the Sr\textsuperscript{85} collected from the femoral vein corrected for the additional Sr\textsuperscript{85} which bypassed this vein. The equation used was:

\[
\text{Recovery of Sr\textsuperscript{85}} = U_n + \frac{(%\ dose\ Sr\textsuperscript{85}\textsubscript{f v})}{ER}
\] (3)

Bone Clearance of Sr\textsuperscript{85}

Because of the rapidly changing concentration of isotope in blood, clearance must be calculated on a dynamic basis. Assuming that the clearance, \(C\), by any bone remains essentially constant during the clearance period, the uptake of Sr\textsuperscript{85}, \(dU\), in an infinitely short period, \(dt\), at a blood concentration of \(S\) will be given by the equation:

\[
dU = C.S.dt
\] (4)

Integrating this expression for the five-minute clearance period will give:

\[
\int_0^5 dU = C \int_0^5 S.dt = U_n \text{ (total bone uptake of isotope in 5 min)}
\] (5)
TABLE 1

Extraction Ratio for Uptake of Sr** by Tibia in the Dog

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Body weight kg</th>
<th>Evans blue %</th>
<th>Sr** %</th>
<th>Extraction ratio (a) - (b) (a)</th>
<th>Bone uptake of Sr** % dose (c)</th>
<th>Sr** recovery % (b) X 100 + (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>73.3</td>
<td>21.5</td>
<td>0.70</td>
<td>68.0</td>
<td>97.3</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>83.3</td>
<td>23.3</td>
<td>0.70</td>
<td>65.1</td>
<td>97.9</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>95.4</td>
<td>10.5</td>
<td>0.89</td>
<td>73.5</td>
<td>94.5</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>91.6</td>
<td>20.5</td>
<td>0.78</td>
<td>69.5</td>
<td>91.8</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>91.1</td>
<td>18.3</td>
<td>0.82</td>
<td>78.3</td>
<td>96.1</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>71.8</td>
<td>14.3</td>
<td>0.80</td>
<td>72.7</td>
<td>92.6</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>98.0</td>
<td>19.7</td>
<td>0.79</td>
<td>70.8</td>
<td>90.9</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>83.2</td>
<td>25.5</td>
<td>0.70</td>
<td>70.8</td>
<td>101.4</td>
</tr>
<tr>
<td>9</td>
<td>22</td>
<td>93.6</td>
<td>26.8</td>
<td>0.71</td>
<td>71.4</td>
<td>100.0</td>
</tr>
<tr>
<td>10</td>
<td>23</td>
<td>91.3</td>
<td>23.8</td>
<td>0.72</td>
<td>69.0</td>
<td>95.0</td>
</tr>
</tbody>
</table>

Average 21.5 ± 9.08 20.22 ± 5.15 0.764 ± 0.066 70.91 ± 3.53 94.75 ± 4.97

*Percentage of injected dose found in the femoral venous blood collected for the initial five minutes immediately following the injection.
†Percentage of injected dose.
SD, standard deviation.

\[
\text{clearance } C = \frac{U_n}{\int_0^5 S \, dt} \quad (6)
\]

where \( \int_0^5 S \, dt = \) the integrated area under the curve of Sr** activity plotted against time (fig. 2). This value may be determined from the graph. When divided by 5, it will equal the average concentration of Sr** in 1 ml blood during the clearance period.

In view of this, the determination of clearance can be greatly simplified by withdrawing blood from the carotid artery during the clearance period at a constant rate, using a constant rate withdrawal syringe pump.* This sample is then thoroughly mixed and the Sr** concentration (X dose/ml) is determined on a 1 ml aliquot to give the average specific activity during the clearance period. This, divided into the bone uptake, will give the clearance for the five-minute period from which the clearance in ml per minute can be calculated readily. Clearance determinations were done on 10 dogs weighing 11.0 to 25.0 kg.

Similar experiments were done on 80 rabbits weighing 1.5 to 3.5 kg. These animals were fasted overnight, and were anesthetized by intraperitoneal injection of 1 g urethane and 250 mg barbital/kg body weight. The carotid artery was cannulated and connected to the continuous withdrawal syringe pump, which was used to remove blood samples immediately after the dose of 10 μC/kg of Sr** in 1 ml saline was injected rapidly into the ear vein. The other procedures were as described above.

Results and Discussion

A. Determination of Sr** Extraction Ratio

Data and calculations of Sr** extraction ratio are given in tables 1 and 2 and illustrated in figure 1. As indicated in table 2, over 80% of the injected plasma dye was collected from the femoral vein in the first two minutes and over 90% in the first five minutes. Thus most of the blood entering the nutrient artery of the tibia returned to the body by this route. The remaining 10% may represent blood which bypassed the femoral vein and returned by other channels. The values agree well with the data of Post and Shoemaker on recovery of blood labelled with Cr** tagged red cells or T-1824 which had been injected into the nutrient artery of the femur in dogs. They collected 73 ± 12% of the labelled blood from the upper and lower venous efflux systems. However, they pointed out that their method

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*American Instrument Company, Silver Springs, Maryland. Catalogue no. 5-8292.
did not collect blood leaving the bone through the small periosteal vessels or through the nutrient vein.

The extraction ratio (ER) was calculated on the assumption that the Evans blue which was not collected from the femoral vein represented venous outflow from bone (with similar Sr\(^{85}\) concentration) which had returned to the circulation by other channels. It is possible that the missing Evans blue may have been retained in bone, so that the blood collected from the femoral vein would represent a higher proportion of the labelled blood which had entered the nutrient artery. If all the missing Evans blue had been retained in bone, and all the blood entering the nutrient artery had been collected from the femoral vein, equation 1 would be used and the extraction ratio would be 0.798 rather than the more conservative figure of 0.764 based on the calculations used in this paper.

The calculated values for the extraction ratio for the five-minute period were very consistent for such measurements, with a standard deviation of less than ± 9%. When calculated on the basis of one-minute collection periods (table 2) the highest value was obtained for the first minute, and for the sample containing the largest fraction of the dye and isotope. The extraction ratios for the later periods were somewhat lower, but the absolute values were so small that the calculation was not nearly as accurate as that based on the entire five-minute period.

A number of factors may account for the finding that the removal of Sr\(^{85}\) by bone was not complete and the extraction ratio was less than 1.0. The nutrient artery supplies both bone tissue and bone marrow, and some flow through the marrow sinuses may not come into intimate contact with mineral. The exchange between Sr\(^{85}\) and the nonradioactive calcium on the surface of the bone mineral may not be 100% during the time of transit, although it must approach this value. Another factor would be the gradual accumulation of Sr\(^{85}\) in the labile calcium pool in bone with which the blood comes into equilibrium. This pool has been estimated at 40 to 80 mg Ca per kg in man.\(^7\) From data on the accumulation of Sr\(^{85}\) in bone, it is estimated that the error introduced by this factor would be less than 5% for the initial five-minute clearance period, but might reach as high as 15 to 20% for a ten-minute clearance period. Obviously, the longer the period of Sr\(^{85}\) accumulation in the skeleton, the higher will be the isotope concentration in this pool and in the venous blood. A shorter clearance period (e.g. 0 to 2 minutes) might reduce this source of error almost entirely.

The extraction ratio for Sr\(^{85}\) removal by bone (0.764 ± 0.021) compares favorably with values reported for removal of bromsulphophthalein (BSP) by liver\(^8\) (0.34 ± 0.12), but is not as satisfactory as those obtained for extraction of diodrast\(^7\) (0.79 to 0.96) and PAH\(^5\) (0.81-0.96) by kidney. This is not surprising in view of the possible blood flow through shunts and in bone marrow. However, the

### Table 2

<table>
<thead>
<tr>
<th>Time interval</th>
<th>Percentage of injected Evans blue (a)</th>
<th>Percentage of injected Sr(^{85}) (b)</th>
<th>Sr(^{85}) extraction ratio (a) - (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>63.2</td>
<td>12.8</td>
<td>0.797</td>
</tr>
<tr>
<td>0-1</td>
<td>18.4</td>
<td>5.5</td>
<td>0.739</td>
</tr>
<tr>
<td>1-2</td>
<td>4.9</td>
<td>2.0</td>
<td>0.633</td>
</tr>
<tr>
<td>2-3</td>
<td>4.1</td>
<td>1.1</td>
<td>0.786</td>
</tr>
<tr>
<td>3-4</td>
<td>2.0</td>
<td>0.6</td>
<td>0.680</td>
</tr>
<tr>
<td>4-5</td>
<td>22.0</td>
<td>22.0</td>
<td>(average 0.762)</td>
</tr>
</tbody>
</table>

*Values are the averages for the five dogs studied.

\(^7\)Bone uptake was 71.3%; estimated recovery 95.1%.
data for bone are remarkably consistent, and in view of this, we believe that the initial (0 to 5 minute) Sr$^{85}$ clearance should give a valid and useful measure of the effective blood flow through those regions in bone where active mineral exchange is taking place. This would be the minimal value. Total blood flow through the bone could be estimated by dividing this by the extraction ratio.

8. ESTIMATION OF INITIAL Sr$^{85}$ CLEARANCE

In eight dogs, the initial Sr$^{85}$ clearance by femur ranged from 3.0 to 18.0 ml/min, and when corrected for the extraction ratio, the range of total bone blood flow was 4.2 to 25.2 ml/min. These values agree very well with those obtained by Post and Shoemaker who used a direct method of collecting the venous outflow from this bone. The more significant values are those computed on the basis of fresh bone weight, and these are given for a number of different bones in adult dogs and rabbits in table 3. There is surprising uniformity of blood flow through these various bones. The values are somewhat higher than those obtained by Weinman et al. using a similar clearance technique. However, they used a ten-minute clearance period which would tend to give lower values. Blood flow through bone equals or exceeds the flow through resting skeletal muscle (1.8 to 9.6 ml/100 ml muscle per minute) reported by Cooper et al. The high flow rate through bone is particularly remarkable in view of low metabolic requirements of this tissue which contains relatively few cells. In our view, this is related to the homeostatic function of bone as a mineral reservoir stabilizing ion levels in blood, and is analogous to the high rate of renal blood flow which far exceeds the metabolic requirements of the kidney.

The effective blood flow through the entire skeleton was estimated for each animal on the assumption that it was similar to that in the bones studied, and that the skeleton comprised 10% of the body weight. This blood flow was then compared to the resting cardiac output calculated from the formula of Smith et al. (resting cardiac output = 182 ± 19 ml/kg) for the dog. For the 10 dogs, the skeletal blood flow was 5.32 ± 2.15% (sd) of the resting cardiac output, which was comparable to the values (5.2 to 6.9%) reported by Weinman et al. and by Ray et al. (3.5 to 9.3%). Correcting for extraction ratio, this would mean that total skeletal blood flow amounted to 7.45 ± 3.02% (sd) of the resting cardiac output, and so comprises a significant portion of the peripheral circulation.

Summary

When a nondiffusible plasma dye (T-1824) and Sr$^{85}$ were injected into the nutrient artery of the tibia in 10 dogs, 90% of the T-1824 and 21% of the Sr$^{85}$ were recovered from the femoral vein in the next five minutes. From these data, the extraction ratio for removal of Sr$^{85}$ was calculated to be 0.764 ± 0.066 (sd). The difference from unity may be accounted for...
in part by blood flow through bone marrow. It was concluded that initial (0 to 5 minute) Sr\textsuperscript{4+} clearance should give a useful measure of effective bone blood flow. Values obtained for femur, tibia, humerus, and talus in 10 dogs and 80 rabbits were all remarkably similar. The effective bone blood flow in all cases was approximately 10 ml/100 g/min or, for the entire skeleton, approximately 5 to 7\% of the resting cardiac output. The rate of blood flow far exceeds the metabolic needs of bone. This may be related to its important homeostatic function in mineral metabolism.

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References
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