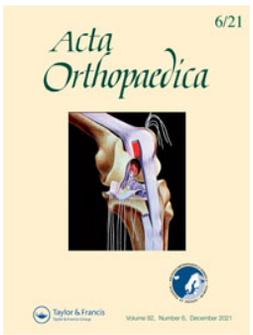

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REGIONAL VASCULAR VOLUMES AND DYNAMIC HAEMATOCRIT COMPARED TO REGIONAL PERFUSION IN CANINE CANCELLOUS AND CORTICAL BONE

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Regional vascular volumes in different areas of long bones in dogs were measured with ^{125}I-fibrinogen and ^{51}Cr erythrocytes. According to the volumes determined, the dynamic small vessel haematocrit was calculated to be 50 per cent of the arterial haematocrit in cancellous bone and 75 per cent of the arterial haematocrit in cortical bone.

The perfusion rate in the same regions was determined with ^{99}Tc^{m}-labelled microspheres (size 15 $\mu \pm 5 \mu$). A linear relation between perfusion rate and blood volume was demonstrated. The data obtained showed that the perfusion rate and blood volume in the red marrow in the femoral neck are 25 times greater than values obtained from tibial and femoral cortical bone.

A thorough testing of the plasma indicators showed that the distribution volume in the tissue for albumin and transferrin is twice the value determined with fibrinogen. The egress of the indicators seems to be correlated with molecular weight.

Key words: bone blood flow; microcirculation

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Data concerning the vascular space and information regarding the ratio between red cells and blood plasma would increase our knowledge of circulatory phenomena in long bones. Brookes (1971) has provided information concerning blood flow rate, erythrocyte flow rate and tissue haematocrit in rat femoral bone circulation. Tøndevold & Eliasen (1982) have demonstrated large blood flow differences in separate anatomical regions in the long bones of dogs.

The purpose of the present study is to determine plasma and erythrocyte volumes in different areas of the long bones in dogs, and to calculate the dynamic small vessel haematocrit in order to evaluate a possible correlation of this parameter with regional blood flow.

MATERIAL AND METHODS

Surgical and experimental procedure

Six adult mongrel dogs (with closed epiphyseal lines, weight 20–30 kg) were premedicated with 2-propyl-10 (dimethyl-aminopropyl)-fluthiazid 0.05 ml/kg (Combelene$^{(R)}$) and anaesthetized with thiomebumal sodium (Leopenthal$^{(R)}$) 12.5 mg/kg. The anaesthesia was maintained with Halothane$^{(R)}$/N_2O/O_2, and the dogs were ventilated with a constant volume respirator after oro-tracheal intubation. Muscle relaxation was obtained with intermittent doses of pancuronium bromide (Pavulon$^{(R)}$). The dogs were placed on a heated operating table and the temperature kept constant with the aid of a rectal thermistor.

After bladder catheterization the exposed right brachial artery was cannulated with a polyethylene catheter. The left carotid artery was exposed and a pig tail catheter no. 8 F was introduced into the left ventricle. The right external jugular vein was isolated and a flow directed Swan-Ganz thermodilution catheter was

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introduced into the pulmonary artery. Through an upper median incision the spleen was removed. ECG lead 2 was monitored. Mean arterial and pulmonary arterial pressures were measured with Statham 23b transducers and S&W amplifiers and digitally displayed with heart rate and temperature. The ECG and pressure curves could if necessary be recorded on an Ultralette(R) strip chart recorder. The cardiac output was measured with a thermodilution technique with a modified thermodilution computer (Devices, USA). After surgery the dogs received 5000 I.U. Heparin Leo(R) subcutaneously and 5000 I.U. Heparin Leo(R) intravenously, whereafter at least 30 minutes was allowed for recovery during which cardiac output, mean arterial pressure, pulmonary pressure, heart rate, arterial blood gases and arterial acid-base balance were monitored.

Microspheres were injected into the left ventricle through the pig tail catheter by means of a Krogh syringe, 5 ml of the suspension in 10 seconds, and flushed with 3 ml saline. Fifteen seconds before injection a suction pump (Braun, Melsungen, Germany) was started and a reference blood sample was obtained from the right brachial artery at a speed of 5 ml/min for 3 minutes. The ^{51}Cr-labelled erythrocytes and ^{125}I-fibrinogen were injected into the pulmonary artery after cessation of collection of the reference sample. After an equilibrium period of 10 minutes, where the variation in all haemodynamic parameters was within 10 per cent, the dogs were killed with a saturated dose of KCl. Immediately the tibial and femoral bones were isolated and all soft tissue removed including the periosteum. The bones were wrapped in water-tight plastic bags and immersed in a mixture of dry ice and hexane and frozen at a temperature of –70°. According to differences in function and metabolic needs biopsies of about 2 g were taken from the bones with a saw (Figure 1). The biopsies were placed in pre-weighed plastic vials for counting of gamma radiation in a well-type counter.

^{51}Cr-labelling of canine erythrocytes

Conventional ^{51}Cr-labelling of canine erythrocytes could not be used as the addition of buffer solution and the multiple washings caused haemolysis. The following technique was used. Forty ml of arterial blood (anticoagulated with citrate) was centrifuged at 300 G for 15 min. The plasma was removed and the buffy coat discarded. Six hundred μCi of Na_2 ^{51}Cr O_4 (Radiochemical Centre, Amersham) was added and the mixture was incubated for 30 min at 37° Celsius. The erythrocyte suspension was washed twice in its own plasma and centrifuged at 300 G for 15 min between the washings. The final suspension contained microscopically normal erythrocytes. After removal of the spleen there was no disappearance of ^{51}Cr activity for 2 hours, and less than 1 per thousand of the ^{51}Cr activity was located in the free blood plasma. It should be mentioned that ^{51}Cr-labelling did not alter the mechanical properties of the erythrocyte membrane, expressed as osmotic resistance of the erythrocytes. The resistance did not differ before and after labelling and it was equal to resistance curves found with human erythrocytes (Tøndevold & Eliasen, unpublished observation).

Analyses

All gamma emitting isotopes were counted in a well-type counter (modified Searle 1195) and all isotopes were separated in a four channel system according to the different levels of their photopeak (^{125}I: 35 KeV, ^{99}Tc^{m}: 140 KeV, ^{113}In^{m}: 393 KeV, ^{51}Cr: 320 KeV). The activity in the samples was corrected for decay in the counting period.

All blood gas analyses were performed on a Radiometer ABL II acid-base laboratory(R).

All haematocrits were determined on a haematocrit centrifuge and centrifuged at 3000 G. No correction for trapped plasma was made.

Labelling of microspheres

Human albumin microspheres (TCK-5-S, Cis, Italy, size 15 μm ± 5 μm) were labelled with 500 μCi ^{99}Tc^{m} in saline according to the instructions given by the manufacturer. The number of spheres injected was approximately 1.5×10^7. All spheres were injected into the left ventricle.

Calculations

The blood flow rate in the tissue (ml·(100 g)$^{-1}$ × min^{-1}) was calculated after correction for the physical decay during the counting period from the formula:

$$\text{Blood flow} = \frac{\text{activity} \cdot (100 \text{ g tissue})^{-1} \times \text{suction pump flow of reference sample}}{\text{total activity in reference sample obtained from suction pump.}}$$

The plasma and erythrocyte volumes in the tissue were calculated from the formula:

$$\text{Plasma volume} = \frac{\text{activity in tissue} \cdot (100 \text{ g})^{-1}}{\text{activity} \cdot (\text{ml reference plasma from left ventricle})^{-1}}$$

$$\text{Erythrocyte volume} = \frac{\text{activity in tissue} \cdot (100 \text{ g})^{-1}}{\text{activity} \cdot (\text{ml of erythrocytes from left ventricle})^{-1}}$$

The tissue haematocrit was calculated from erythrocyte and plasma volume determinations from the formula:

$$\text{Tissue haematocrit} = \frac{\text{erythrocyte volume}}{\text{erythrocyte volume} + \text{plasma volume}} \times 100 \text{ per cent}$$

Experimental considerations concerning the use of plasma indicators in the determination of plasma volumes in bones

The ideal plasma indicator is evenly distributed in the plasma and does not egress to extravasal space. The most commonly used plasma indicators are albumin and $^{113}In^{m}$-labelled transferrin. However, it is known that extravasation of albumin occurs to some extent, but the exact overestimation when using these indicators has not been studied in detail. For non-polar indicators the egress must be linked to molecular weight and to the form of the molecule.

The diffusion coefficient was determined in agar gel for $^{99}Tc^{m}$-albumin (TCK-2, Cis, Italy), for $^{113}In^{m}$-transferrin after labelling using a $^{113}In^{m}$-generator (Radiochemical Centre, Amersham), and for ^{125}I-fibrinogen (Radiochemical Centre, Amersham). The diffusion coefficients and molecular weights are listed in Table 1.

All plasma indicators were injected into the pulmonary artery and the activity in plasma was followed together with the activity of ^{51}Cr-labelled erythrocytes. The ^{125}I-fibrinogen content in blood seems strictly related to the plasma phase as there was a constant concentration in plasma for a 2-hour period after heparinization.

Tissue activity at the time of sacrifice was calculated for all samples using the plasma from blood taken from the left ventricle as reference. The biopsies were taken according to Figure 1. The plasma volumes determined in the same tissue biopsies are shown in Table 2 and it is evident that in a structure such as cancellous bone the egress of albumin and transferrin is considerable. In brain where the passage of solutes from the circulating blood to the tissue is quantitatively different because of the blood-brain barrier, the plasma volumes determined were equal for the three indicators used. There was no difference in the determinations of tissue plasma volumes with regard to the time of equilibration after injection of the indicators.

This means that the egress of albumin and transferrin takes place within 10 minutes after the injection (Table 2).

The impurities in the commercially available ^{125}I-fibrinogen are about 5 per cent and consist mainly of free iodine. To elucidate the importance of this the fibrinogen was passed through Millipore$^{(R)}$ filters allowing all molecules with a molecular weight below 10^4 $g \cdot mol^{-1}$ to pass. Free iodine was rinsed from the fibrinogen by ultrafiltration through a Millipore$^{(R)}$ filter (10^{-4}), using 2 ml of sterile saline and about 1 atmosphere pressure. By repeating this procedure 7 to 10 times the amount of free ^{125}I decreased to 5 per thousand (Sejersen 1976).

The plasma volumes determined in the bones with this rinsed plasma indicator were the same as with the unrinsed commercially available ^{125}I-fibrinogen (Radiochemical Centre, Amersham). We therefore selected ^{125}I-fibrinogen as our plasma indicator.

Table 1. The diffusion coefficient and molecular weight of the indicators used. All diffusion coefficients are expressed as cm^2/s and all molecular weights are in g/mol

Indicator	Diff. coeff.	Mol. weight	% Impurities
^{125}I-Fibrinogen	8.5×10^{-8}	340,000	5%
$^{113}In^{m}$-Transferrin	5.3×10^{-7}	57,000	50%
$^{99}Tc^{m}$-Albumin	3.4×10^{-7}	69,000	5%

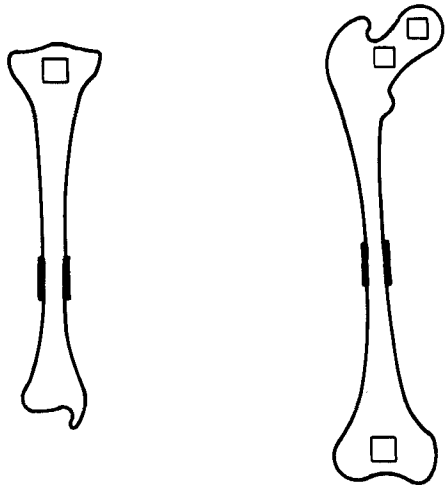

Figure 1. The anatomical location of the biopsies taken from the tibia (left) and femur (right).

RESULTS

The regional perfusion rates in different areas of the long bones are shown in Figure 2 and Table 3.

Table 2. The distribution of the three indicators tested in the study in different parts of the long bones

Location of biopsy	$^{99}Tc^{m}$-Albumin ml (100 g)$^{-1}$±1.s.d.	^{125}I-Fibrinogen ml (100 g)$^{-1}$±1.s.d.	$^{113}In^{m}$-Transferrin ml (100 g)$^{-1}$±1.s.d.
Femoral head $n=5$	6.37±2.67	3.05±0.43	7.77±1.18
Femoral neck $n=6$	11.52±3.08	8.74±1.85	19.40±2.41
Cortical bone $n=6$	1.64±1.14	0.51±0.20	1.17±0.37
Brain $n=10$	0.68±0.0	0.68±0.0	0.69±0.0

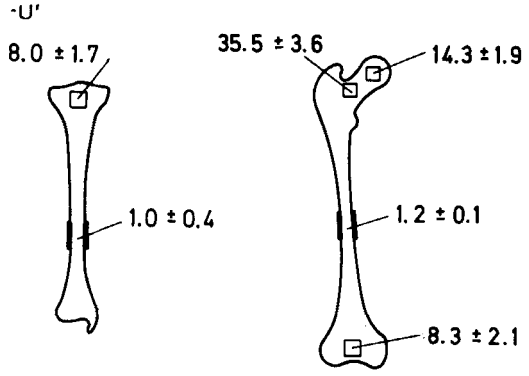

Figure 2. The perfusion rates in different anatomical areas in long bones determined with $^{99}Tc^{m}$-labelled human albumin microspheres. All values are given in ml blood (100 g)$^{-1}$ × min^{-1} ± 1 s.e.m.

The cortical flow was found to be 1.2 ml·(100 g)$^{-1}$ × min^{-1} in both hind limb bones. The fat-filled metaphyseal cancellous bone above and below the knee showed a perfusion rate of 8.3 and 8.0 ml·(100 g)$^{-1}$ × min^{-1} in the femur and tibia, respectively. In the femoral neck the perfusion rate was determined to be 35.5 ml·(100 g)$^{-1}$ ×

Table 3. The perfusion rates in different areas of long bones measured with $^{99}Tc^{m}$-labelled human albumin microspheres

Location of biopsy	Blood flow rate ml (100 g)$^{-1}$×min^{-1} mean ± 1 s.e.m.
Femoral head	14.3±1.9 ($n=12$)
Femoral neck	35.5±3.6 ($n=12$)
Femoral cortical bone	1.2±0.1 ($n=12$)
Supracondylar cancellous bone	8.3±2.1 ($n=12$)
Tibial condylar cancellous bone	8.0±1.7 ($n=12$)
Tibial cortical bone	1.0±0.4 ($n=12$)

Table 4. The distribution volumes of ^{125}I-fibrinogen as plasma indicator and ^{51}Cr-labelled erythrocytes as erythrocyte indicator in different regions in long bones. Blood volume is calculated from the erythrocyte and plasma volumes, and haematocrit in the tissue is determined using the volumes found with the erythrocyte and plasma indicator. Note that all values are normalized to an arterial haematocrit of 40 per cent

Location of biopsy $n=12$	Plasma vol. ml (100 g)$^{-1}$ ±1.s.d.	Erythrocyte vol. ml (100 g)$^{-1}$ ±1.s.d.	Blood vol. ml (100 g)$^{-1}$ ±1.s.d.	Tissue Haematocrit in %
Femoral head	2.85±0.46	0.73±0.10	3.58±0.51	21±2
Femoral neck	5.88±1.20	1.39±0.28	7.27±1.43	19±2
Femoral cortical bone	0.21±0.07	0.09±0.03	0.30±0.10	29±3
Femoral supracondylar bone	2.57±2.15	0.70±0.43	3.22±2.62	22±4
Tibial condylar cancellous bone	1.94±2.05	0.53±0.55	2.49±2.56	21±3
Tibial cortical bone	0.24±0.06	0.12±0.04	0.36±0.10	32±3

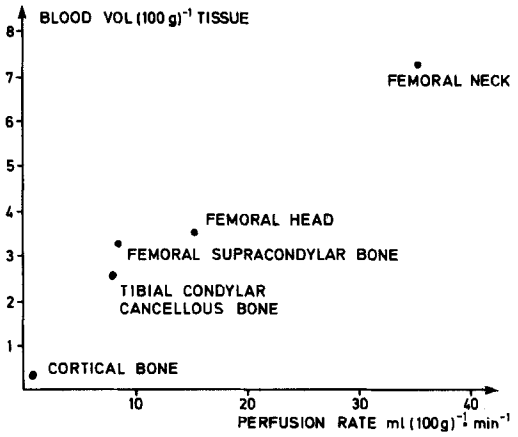

Figure 3. The relation between blood volume and perfusion rate is shown. The blood volume is determined with ^{51}Cr-labelled erythrocytes and ^{125}I-fibrinogen. The perfusion rates are determined with a $^{99}Tc^{m}$-microsphere technique.

min^{-1} and in the femoral head 14.3 ml · (100 g)$^{-1}$ × min^{-1}.

The blood volumes determined showed a similar pattern (Table 4). The cortical bones contained approximately 0.3 ml · (100 g)$^{-1}$ blood with a haematocrit of approximately 31 per cent compared to 40 per cent in arterial blood. The femoral neck contained 7.3 ml · (100 g)$^{-1}$ blood with a haematocrit of 19 per cent.

In the femoral head the blood volume was determined to be 3.6 ml · (100 g)$^{-1}$ with a haematocrit of 21 per cent. Similar values were determined for cancellous bone on both sides of the knee. According to Figure 3 a linear relation between perfusion rates and blood volumes was found.

In Table 4 all values are normalized to an arterial haematocrit value of 40 per cent. The arterial haematocrit in the dogs used was in the range of 33 to 48 per cent. It is obvious that this will affect the variation in the tissue haematocrit. Assuming that a linear relation exists or that a certain increase or decrease in arterial haematocrit will induce an equal relative change in tissue haematocrit, all values are corrected for this experimentally induced variation. The raw data, however, showed the same mean values but with a greater inter-individual variation.

DISCUSSION

Brookes (1968) suggested that growth, repair and local variations in bone turnover are linked with changes in blood flow. During normal physiological conditions the perfusion rate in cortical and cancellous bone differs (Brookes 1968, Morris & Kelly 1980). Regional differences in long bone perfusion have been described (Tøndevold & Eliasen 1982). Vascularity must thus be considered to be uneven in marrow versus cortical bone and this suggests that metabolic needs are different in the respective areas. To obtain reproducible data concerning measurements of plasma and erythrocyte volumes in bone it was necessary to test the indicators used.

Sixteen per cent of total albumin content in bone is intravascular (Owen & Trifitt 1976). This indicates that albumin egresses through the capillary wall and therefore is unsuitable as a plasma indicator. In the present study it is clearly shown that albumin crosses the membrane of the small vessels. Even a short time of equilibration, in this case 10 minutes, did not reduce the egress. Plasma volume determination in comparable parts of the long bones showed great variations with the indicators used, having different diffusion capacities.

The diffusion coefficient is among other things linked to the molecular weight. In brain tissue, which in the present study serves as a biological control, all plasma volumes were found to be equal indicating that the distribution of the three indicators in the vascular space was uniform. ^{125}I-fibrinogen was in this study shown to be the most suitable indicator for volume determination in the tissues. This finding is in accordance with results obtained by Bell et al. (1980) and Watson et al. (1980). They have studied ratios between albumin and fibrinogen in plasma and lymph. Their experiments showed the egress of albumin as lymph to tissue ratio is 0.24 and that for fibrinogen 0.04, in lymph collected from mixed tissue in the hind paw in dogs. As the molecular weight of $^{113}In^{m}$-transferrin is in the same range as albumin a corresponding egress would be expected. The plasma volumes in tissue detected with this indicator are the same as with albumin. All this favours fibrinogen as a plasma indicator

in a heparinized animal when measuring plasma volume in tissue. The low energy (35 KeV) of ^{125}I-fibrinogen is a factor of interest in the counting procedure.

In a pilot study no detectable attenuation through pieces of cortical bone could be found in the samples (Tøndevold & Eliasen, unpublished observation).

The ^{51}Cr activity in blood was closely related to ^{51}Cr-labelled erythrocytes. Less than 1 per thousand of the ^{51}Cr activity was found in plasma, and after removal of the spleen, no disappearance of ^{51}Cr activity from the blood during the experiment was found. The small vessel haematocrit value based on erythrocyte and plasma volume determinations is in accordance with observations made by Johnson et al. (1971) and Klitzman & Duling (1979). They studied capillary volume and erythrocyte velocity and were able to calculate capillary haematocrit in the mesenterium and cremaster muscle, respectively. Brookes (1971) collected blood from the nutrient vein in rat tibias after ^{51}Cr-labelling of the erythrocytes. He found a haematocrit which was greater in the nutrient vein than in the arterial circulation. He detected differences in ^{51}Cr activity in separate parts of the femoral bones indicating differences in vascularity. Brookes' determination of haematocrit in various rat bones could, however, not be compared with ours as his estimation is related to the haematocrit in the nutrient vein.

It is an open question whether or not bone contains lymphatic channels (Seligher 1970). The amount of water removed by the lymphatic channels would have to be considerable in order to explain the haemoconcentration in the nutrient vein found by Brookes. However, Brookes' relative haematocrits are similar to ours. It is unlikely that the microspheres would interfere with the volumes measured. According to Hales (1974) only 0.01 per cent of the capillaries are blocked by a single injection of spheres.

Johnson et al. (1971) found a haematocrit in the mesenterium which was around 30 per cent of the arterial value when looking directly at the capillaries. Jodal & Lundgren (1970) found values in intestinal mucosa close to ours and they used a similar technique. Our tissue represents all vessels larger than 100 μm in diameter. We determined the small vessel haematocrit while Johnson et al. (1971) studied the capillaries. At present it is not possible to reach a higher degree of differentiation in the microcirculation in bone. Due to differences in capillary transit time it is possible that the haematocrit in single capillaries is below the values determined in the present study.

Jodal & Lundgren (1970) also detected that the mucosal haematocrit rose to arterial values when the flow in the mesenterium was reduced. This is in accordance with our findings that an increased haematocrit is present in areas with a low perfusion. The haematocrit found in cortical bone is considerably larger than haematocrits found in cancellous bone.

Using a vital microscopic method Brånemark (1959) found higher erythrocyte velocities in cortical bone compared with red marrow. The higher haematocrit might explain this, as a relatively greater number of erythrocytes have to pass through the vessel at the same blood flow rate and the same vascular volume, resulting in an increased linear velocity of the erythrocytes. It is interesting to note that cancellous bone has a perfusion rate which is 30 times higher than cortical bone resulting in a much higher oxygen supply to the cancellous bone in spite of the lower haematocrit in this region, presumably reflecting different metabolic demands.

The blood flow rates measured in this study are lower than the values obtained in an earlier study (Tøndevold & Eliasen 1982). This can be explained by the age of the dogs used. All our dogs were mature with closed epiphyseal lines. Brookes (1965) and Whiteside et al. (1977) found reduced blood flow in the bones of aged animals. Perfusion seems related to osteoblast activity (Whiteside et al. 1977). In the present study the blood flow in the distal femoral metaphysis is low as the marrow in this region is fatty. The perfusion rate in cortical bone is about the same as in adipose tissue.

In the measurements of bone vascularity the present investigation clearly shows:

1) A linear relation exists between perfusion rate and blood volume. The regional differences in vascular volumes are proportional to differ-

ences in blood flow rate. The volume of the vessels in cancellous bone is 25 times larger than in cortical bone.

2) The dynamic small vessel haematocrit is 75 per cent of the value in the left ventricle in cortical bone and 50 per cent in cancellous bone.
3) The distribution volume for plasma indicators shows that with indicators with a molecular weight below 10^5 g/mol the volume determined is twice that obtained with an indicator with a molecular weight of 4×10^5 g/mol.
4) The egress of indicators with a molecular weight below 10^5 g/mol takes place within 10 minutes after the injection of the tracer.

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