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# BLOOD FLOW RATES IN CANINE CORTICAL AND CANCELLOUS BONE MEASURED WITH $^{99}Tc^{m}$-LABELLED HUMAN ALBUMIN MICROSPHERES

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Regional differences in bone blood flow rates in the femur and the tibia of dogs were measured with $^{99}Tc^{m}$-labelled microspheres. The measurements show an average flow rate of 3.7 ml $(100\ g)^{-1} \times min^{-1}$ in cortical bone. A more rapid pace was found in red marrow containing areas, with an average flow rate in the femoral head of 19.7 ml blood $(100\ g)^{-1} \times min^{-1}$, and in the femoral neck of 50.3 ml blood $(100\ g)^{-1} \times min^{-1}$. In the calcar femorale the average flow rate was 9.0 ml $(100\ g)^{-1} \times min^{-1}$, and compared to cortical flow a positive correlation between strain and perfusion seems obvious. No difference between cortical bone in tibia and femur was found. The flow rates in the red marrow of the femoral neck are remarkably high, but the flow in the cortical bone is relatively low.

It is concluded that handling of fragments of cortical bone and the associated soft tissue is presumably critical, and that the surgical technique has to be quite gentle to obtain optimal conditions for fracture healing.

*Key words:* bone blood flow; microcirculation; microspheres

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Fracture healing depends on the viability of bone (Szyszkowitz & Brüggemann 1977), and thus on the perfusion of the tissue, but also other factors contribute to the repair processes of broken bones (Hulth 1980).

Quantitative measurements of blood flow in cortical and cancellous bone have been difficult to perform due to the disperse nature of the arterial inflow and the rigid tissue structure.

Brånemark (1959) measured erythrocyte velocity in bone marrow by vital microscopy. Cumming (1962) measured bone flow by collecting venous outflow, whereas Brookes (1967) used the transit time of $^{51}Cr$-tagged erythrocytes in his estimation. Copp & Shim (1965) and Shim et al. (1971) used the clearance of bone-seeking radioisotopes.

The introduction of microspheres and their use in circulatory physiology has made perfusion studies of inaccessible organs easy to perform. The validity of this method has been stated by Rudolph & Heymann (1967) and Buckberg et al. (1971).

In bone, Lunde & Michelsen (1970), Gross et al. (1979) and Okubo et al. (1979) have used this technique. Their measurements, however, were not correlated with distinct anatomical and functional regions in bones.

The purpose of this study was to determine flow rates in various regions in tubular long bones, and to elucidate the perfusion of surgically critical areas.

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## MATERIAL AND METHODS

*Surgical and experimental procedure*

Six adult mongrel dogs (with closed epiphyseal lines, weight 20–25 kg) were premedicated with 2-propyl-10 (dimethylaminopropyl) fluthiazid, 0.05 ml/kg (Combelene(R)) and anaesthetized with thiomebumal sodium (Leopenthal(R)) 12.5 mg/kg. The anaesthesia was maintained with $N_2O/O_2$ Halothane (R) 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 catheterisation, the exposed right brachial artery was cannulated with a polyethylene catheter. The left carotid artery was exposed and a pig tail catheter N° 8F was introduced into the left ventricle. The right external jugular vein was isolated and a flow direct Swan-Ganz Thermo-dilution catheter was introduced into the pulmonary artery. ECG lead II was monitored.

Mean arterial pressure and pulmonary arterial pressure were measured with Statham 23b transducers and S&W amplifiers, and digitally displayed with heart rate and temperature. The mentioned parameters and pressure curves could if necessary be recorded on an Ultralette(R) Strip-Chart recorder. The cardiac output was measured by a thermodilution technique with a modified thermodilution computer (Devices, USA).

After surgery the dogs recovered for at least 30 min, during which time cardiac output, mean arterial pressure, pulmonary pressure, heart rate, arterial blood gases and acid-base balance were checked.

Microspheres were injected into the left ventricle through the pig tail catheter by means of a Krogh syringe, 5 ml of the suspension in 15 s and flushed with 5 ml saline.

Thirty seconds before injection a suction pump (Braun, Melsungen, West Germany) was started and reference blood samples were obtained from the right brachial artery at a rate of 5 ml $(min)^{-1}$ up to 15 ml.

After a period of 30 min the dogs were killed by injection of a dose of saturated KCl. The femora and tibiae were isolated and all soft tissue was removed, including the periosteum. The bones were divided with a saw into biopsies of about 1 g, as illustrated in Figure 1. The biopsies were placed in preweighed plastic vials for radioactive counting. All weights were determined on a Mettler precision balance.

*Analyses*

All tissue specimens and blood samples collected from the suction pump were analysed with regard to gamma radiation in a well-type counter, amplifier/discriminator (Sodiumiodide crystal, Meditronic S.H.A. 11). The activity in the samples was counted using different time intervals to avoid dead-time loss.

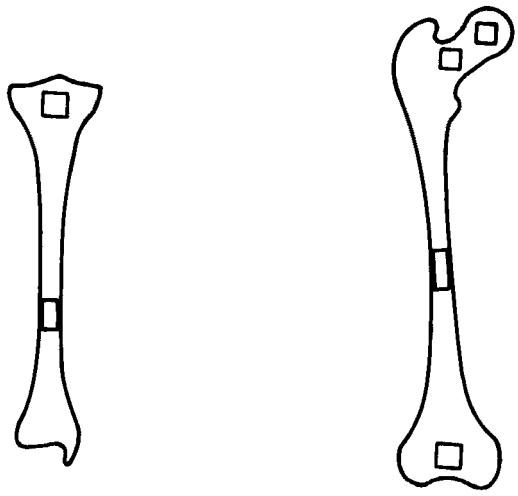

*Figure 1. Anatomical location of biopsy.*

*Preparation and labelling of microspheres*

Human albumin microspheres (TCK-5-S, Italy size 15 μm) were prepared according to the instructions given by the manufacturer and labelled with 4 mCi $^{99}Tc^{m}$. In every experiment $13 \times 10^6$ microspheres were used. Direct microscopy revealed no aggregation of microspheres in the suspension.

*Calculation*

The blood flow rate of the tissue (ml $(100\ g)^{-1} \times min^{-1}$) was calculated, after correction for the physical decay occurring during the counting process, from the formula:

$$\text{Blood flow rate} = \frac{\text{Activity/100 g tissue} \times \text{reference sample ml/min}}{\text{activity of reference sample}}$$

## RESULTS

The regional perfusion in cortical and cancellous bone is presented in Table 1.

As shown, the lowest perfusion rate was found in the cortical diaphyseal bone (3.7 ml $(100\ g)^{-1} \times min^{-1}$). There was no significant difference between femur and tibia even if the tibial biopsy was taken from the junction between the distal one-third and the proximal two-thirds.

*Table 1. The regional perfusion in cortical and cancellous bone*

| Location of biopsy according to Figure 1 | Flow rate ml $(100\ g)^{-1} \times min^{-1}$ mean ± 1 S.E. (12 observations) |
|---|---|
| FEMUR | |
| femoral head | 19.6±1.3 |
| femoral neck | 50.3±4.6 |
| calcar femorale | 9.0±0.8 |
| femoral cortical bone | 3.7±0.8* |
| femoral supracondylar cancellous bone | 27 ±3.5** |
| TIBIA | |
| tibial condylar cancellous bone | 29 ±5.8** |
| tibial cortical bone | 3.7±0.7* |

The data marked with * and ** are not significantly different from each other, but significantly different from the other values using Student's *t*-test.

The perfusion rate of the calcar femorale (9.0 ml $(100\ g)^{-1} \times min^{-1}$) was significantly lower than the perfusion of cancellous bone obtained from the femoral neck (50.3 ml $(100\ g)^{-1} \times min^{-1}$). The flow measured in the femoral neck was the highest obtained and more than twice the flow of the femoral head (19.6 ml $(100\ g)^{-1} \times min^{-1}$). The perfusion of the cancellous bone on both sides of the knee joint showed an intermediate flow (27 and 29 ml $(100\ g)^{-1} \times min^{-1}$).

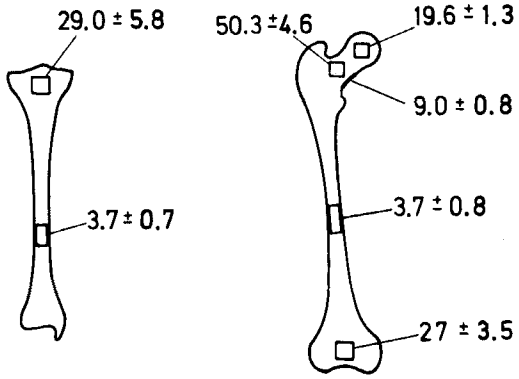


*Figure 2. Perfusion rates in different anatomical areas in long bones. All values in ml blood $(100\ g)^{-1}$ tissue $\times$ $min^{-1}$ ± S.E.M.*

## DISCUSSION

Until now it has not been possible to make non-invasive quantitative measurements of regional cortical or cancellous human bone blood flow.

The venous effluent collection method used by Cumming (1962) necessitated surgical interventions which might interfere with the flow to be measured (Bosch 1969). Flow estimations by clearance of bone seeking radioisotopes, used by Copp & Shim (1965), Shim et al. (1971), Wotton et al. (1976), assume that the tracer is completely cleared in a single passage through the bone, and that no reflux occurs from bone to blood. The latter assumption seems not to be justified (Wotton et al. 1976), and the technique has been claimed to be unsatisfactory compared to the microsphere technique especially at middle and high flow rates (Schoutens et al. 1979).

The $^{133}X^{e}$-deposit washout method used by Lahtinen et al. (1979) might be valuable in determination of cancellous bone flow, but could not be used in cortical bone. There are, however, difficulties in using this method as the tissue homogeneity is uncertain and the tissue trauma might interfere with the washout technique. Further, the partition coefficient of Xenon used was calculated from a tissue haematocrit of 40 per cent. Calculation of dynamic tissue haematocrit, however, gives a value for bone of 20–25 per cent (Tøndevold & Eliasen, in press).

Measurements of bone blood flow in animals with microspheres is easy. The main assumption when using this technique is that corpuscular elements introduced into the arterial circulation are distributed according to the blood flow. When using radiolabelled microspheres, the tissue activity will reflect the arterial inflow when: 1) The microspheres are evenly suspended in the arterial blood. 2) The microspheres are embolised during their first passage through the microcirculation. 3) The injected microspheres have such a quanta that the numbers in single samples exceed a certain minimum, and that they are represented evenly in respect to their mean size. 4) The trapped microspheres do not alter the microcirculation and 5) the major haemodynamic parameters are unaltered (Rudolph & Heyman (1967), Buckberg et al. (1971).

In the present study the microspheres were well suspended after injection into the left ventricle, indicated by differences of about 0.1 per cent in radioactivity of the samples from different parts of the arterial system. The microspheres used had a diameter of 15 μ, and the non-entrapment was found to be 0.3 per cent by sampling from the pulmonary artery during sphere injection.

Buckberg et al. (1971) have outlined the various pitfalls and the basic theory of the use of microspheres. They have stated that at least 400 spheres are necessary in single samples to keep the error for single measurements below 10–15 per cent. This means that in this experiment $13 \times 10^6$ microspheres had to be injected to get 800 microspheres in a tissue sample of 1 g with a blood flow of 12 ml $(100\ g)^{-1} \times min^{-1}$.

With increasing diameter the microspheres are located in the axial stream of the vessels (Phibbs & Dong 1970), as well as their load of radioactivity increases. The particles used by Brookes (1970) and Tothill & McCormick (1976) seem to be of a size which could be located in the central stream in the arteries. This means that the perfusion rate is overestimated in briskly perfused areas. By reducing the particle size to less than 10 μ, their distribution in the arteries is similar to red cells, but the non-entrapment approaches 10 per cent (Crystal et al. 1979).

When making flow studies with microspheres it is essential to maintain the stability of the haemodynamic parameters. It has been shown that intramedullary pressure depends on arterial pressure and arterial oxygen tension (Tøndevold et al. 1979a, b). Variation in flow rates might be due to hypoxaemia or hypotension. The results obtained in this study show a marked difference in regional blood flow, ranging from 3.7 ml $(100\ g)^{-1} \times min^{-1}$ in cortical bone, up to 50.3 ml $(100\ g)^{-1} \times min^{-1}$ in the cancellous red marrow of the femoral neck. This is in accordance with the cortical flow of 2.6 ml $(100\ g)^{-1} \times min^{-1}$ calculated by Brookes (1974), based on erythrocyte velocities (Brånemark 1959). Our results are also equal to values obtained by Morris & Kelly (1980), who found in conscious dogs, a cortical flow rate of 2.5 ml $(100\ g)^{-1} \times min^{-1}$, and a cancellous bone blood flow in the tibia of about 30 ml $(100\ g)^{-1} \times min^{-1}$. On the contrary the cortical flow of 0.9 ml $(100\ g)^{-1} \times min^{-1}$ obtained by Lunde & Michelsen (1970) could be explained by the surgical interventions on the extremity on which they made their observations.

Okubo et al. (1979) have determined the long bone cortical flow to be 3.0–7.0 ml $(100\ g)^{-1} \times min^{-1}$, and the cancellous bone flow to be in the range of 10–20 ml (100 g)–1 $\times$ $min^{-1}$ but with a considerable variation. The cancellous bone blood flow is less than in our findings, but it is obvious that when a metaphyseal biopsy is taken as a circular disc, it will contain different amounts of cortical bone with a low perfusion rate.

There was no difference between femoral and tibial cortical bone blood flow even if the tibial biopsy was taken from the border of the distal one-third and the proximal two-thirds. The perfusion rate of the femoral head was less than in the neck. The surprisingly high flow in the neck might be due to anatomical reasons as many vessels enter the bone in this region.

When discussing fracture healing it is obvious that blood supply to the fragments is a factor of interest. Diaphyseal bone is poorly perfused, and the soft tissue lesion is of utmost importance for the viability of the bone tissue (Szyszkowitz & Brüggemann 1977). The surgical handling of cortical bone should therefore be such that soft tissue lesions are kept to a minimum.

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