# Haemodynamics of Long Bones

An Experimental Study on Dogs

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2) Tøndevold, E., Eriksen, J. & Jansen, E. (1979, a) Observations on long bone medullary pressure in relation to mean arterial blood pressure in the anaesthetized dog.
Acta Orthop. Scand. **50:** 527 - 531.

3) Tøndevold, E., Eriksen, J. & Jansen, E. (1979, b) Observations on long bone medullary pressures in relation to arterial $PO_2$, $PCO_2$ and pH in the anaesthetized dog.
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4) Tøndevold, E. & Eliasen, P. (1982, a) Blood flow rate in canine cortical and cancellous bone measured with $^{99}Tc^{m}$-labelled human albumin microspheres.
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5) Tøndevold, E. & Eliasen, P. (1982, b) Regional vascular volumes and dynamic haematocrit compared to regional perfusion in canine cancellous and cortical bone.
Acta Orthop. Scand. **53:** 197 - 203.

6) Tøndevold, E. & Bülow, J. (1983) Bone blood flow in conscious dogs at rest and during exercise.
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## FOREWORD

The studies presented were started at the Biomechanical laboratory and Department of Orthopedic Surgery T-2, Gentofte Hospital, Copenhagen, Denmark. Without the profound interest of the heads of the department, K. F. Jansen Ph.D, and P.S. Rasmussen Ph.D, the work could not have been performed.

However, I am greatly indebted to the heads of the Department of Cardiology, Gentofte Hospital, P. Eskildsen Ph.D and P. Fritz-Hansen Ph.D., who made the ideas possible. They willingly supplied me with all the materials necessary for the experiments. During the last part of the work, I received much help from the Department of Clinical Physiology, Gentofte Hospital, and was extremely cordially met by the head of the Department M.O. Dyrbye Ph.D.

The last part of the study was performed during my time as research fellow at the Department of Orthopedic Surgery O, Hillerød Hospital and my chiefs K. Dohn MD, K. Rechnagel MD and K. Skou Andersen Ph.D, has with everlasting effort made my working conditions more than perfect.

When among all, an ortopedic surgeon starts with physiological studies, it is necessary with collaborators that modifies and supports during the work. My colleagues J. Eriksen MD, E. Jansen MD, J.E. Pedersen MD, P. Eliasen MD and J. Bülow Ph.D have used many hours during day and night, and their efforts have been more than friendly. During the years of the work I have received intellectual and practical support from the Institute of Medical Physiology B and C, University of Copenhagen.

The staff in the Experimental laboratory, Department of Cardiology, Gentofte Hospital, M. Kærumgaard and Mrs. Lykke Nielsen has shown patience and confidence, and without their spirit the many hours would have been more exhausting than actually.

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Erik Tøndevold

# Contents

# Preface

"There is danger inherent in the mechanical efficiency of our modern methods, danger lets the craftsman forget that union cannot be imposed, but may have to be encouraged. For a bone is a plant, with its root in the soft tissues, and when its vascular connections are damaged, it often requires, not the technique of a cabinet maker, but the patient care and understanding of a gardener".

Girdlestone, 1932.

# Introduction

The physiological mechanisms concerning the circulation in long bones has not received much attention in the past. It is understandable as the bony tissue in itself is difficult to approach with common physiological methods. Its hardness and disperse vascular supply makes measurements difficult. In the last decades a more profound understanding of the individual long bone as a complete organ is taking form.

Long bone has to be considered as a physiological unit containing different tissues with different functions and metabolic needs. As orthopaedic surgery gets more and more sophisticated in its methods, a more complete picture of bone physiology are needed as we have to know the possibilities in the tissue itself. It is important to note that all surgery upon bones should be compatible with its nutrition and not against. In the present work there are several fundamentals which are discussed, as more basic understanding of bone physiology, necessitates the answers of the following questions:

1) Do long bones show autoregulatoric mechanisms of blood flow, and how is their place in the overall circulation?
2) Do long bones react on alternations in the central blood gas composition, and does the gas composition in the interior of the long bones follow the gas tensions found in the central part of the circulating blood?
3) Do regional differences in bone perfusion exist, and is the perfusion rate equal throughout the individual long bone?
4) Is it possible by physiological means generally or locally, to alter bone blood perfusion rate?
5) Does there exist any correlation between the local perfusion rate and blood volume? Are regions supplied mainly with sinusoides caractherized by a high vascular volume and low perfusion rate?
6) Does extravascular space exist in bone or is the interstitial water compartment negligeable?
7) Are the vessels in bone subjected to differences in permeability for different indicators with different molecular weight?

The following will try to make an approach to the answer of these questions.

# Chapter I

## THE ANATOMICAL SUBSTRATE FOR PHYSIOLOGICAL STUDIES

It is axiomatic that sufficient blood and unimpaired blood supply are essentials in bone metabolism, growth and in repair processes. Since the days of Antoine van Leuwenhoeck who as the first looked in a microscope on the surface of bone, great attention has been given to bone vascularity.

According to Rhinelander (1974) the arterial supply to the long bones consists of three primary components:

1) The principal nutrient artery.
2) Metaphyseal and epiphyseal arteries.
3) Periosteal arterioles which supply the peripheral 1/3 of the cortex. The periosteal arteries also reach the cortex through the attachments of ligaments and muscles.

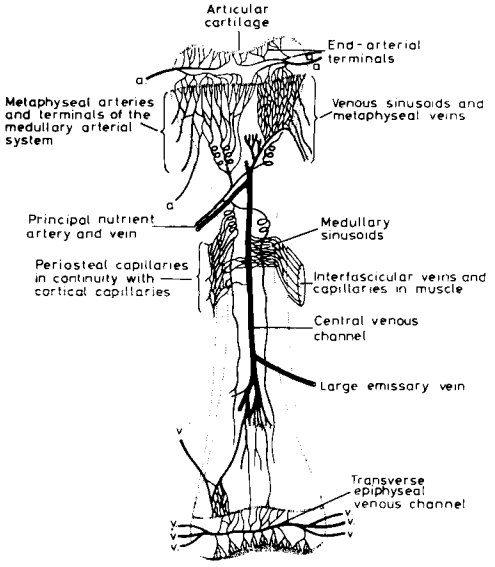


*Figure 1.1*
*The vascular anatomy in long bone according to Brookes (1971).*

According to Brookes (1971) the nutrient artery divides shortly after the passage through the nutrient channel and divides into ascending and descending branches in the marrow cavity. He states that diaphyseal cortical tissue is mainly supplied from medullary vessels. The work of Yoffey (1965) has shown that the arteries in the marrow consist of two types of vessels. He describes a thick-walled and a thin-walled artery. The thick-walled artery is a main branch from the supplying artery and after a short run in the medullary tissue, the wall thins down and consists of two layers of flattened cells. Brånemark (1959) has shown with vital microscopy that this artery divides and forms sinusoides. It should be mentioned that regional differences take place in the architecture and also that the haematopoetic activity of the marrow

observed has great influence on the number of capillaries in the marrow of rabbits.

There exist vessels which leave the marrow and swing into the Haversian channels in the endosteal part of the cortex whereafter they return to venoules in the marrow. Rubascheva and Prives (1932) and Brookes (1971) suggest that many of these small arteries are end-arteries and their findings are confirmed to some degree by Rhinelander (1974). This means that the Haversian channel is to be considered as the only longitudinal vascular channel in the cortex, but does not extend more than a few millimeters before terminating (Cohen & Harris, 1958).

Cooper et al. (1966) have by electron-microscopical methods shown that the capillaries of cortical bone presumably are of a closed continous varity.

The venous outflow in epiphyseal tissue is radial (Brookes, 1971). In metaphyseal and diaphyseal tissue the veins and venoules are abundant, but in contrary to metaphyseal tissue the veins are much larger in diaphyseal tissue and also form a central sinous which drains via the nutrient vein. Yoffey (1965) measured the lumen of the vein and found it more than thirty times larger in crossectional area than the corresponding artery.

The metaphyseal and epiphyseal vessels fuses at maturity. During the growth period they are separated by the epiphyseal plate. In the immature metaphyseal tissue the vessels are dispersed throughout the regions and form a juxtaepiphyseal longitudinal projection of small vessels close to the growth zone. The epiphyseal arteries are radially and transversally grouped with arcades subchondrally (Nussbaum, 1923).

Furthermore, there is an open question if bone contains lymphatic channels (Seligher, 1970) even if most authors at present deny the presence of lymphatic channels in bone (Brookes, 1971).

## PRESENT KNOWLEDGE OF PHYSIOLOGY

Bones are supplied with nerves which possibly take part in the regulation of the perfusion. First observed by Gros in 1846, Ottolenghi in 1902 carried out an extensive work to confirm the existence of both myelinated and non-myelinated fibres in cortical and medullary tissue. Shim (1977) and Duncan & Shim (1975) have by histological methods demonstrated the existence of noradrenergic and cholinergic nerves in the walls of the bone vessels. Nerves are present but to what extent they contribute to the local vasomotion and circulatory regulation in bone is more obscure. It is obvious that selective stimulation of these vasoactive nerves are impossible, and the experiments which have been performed included stimulation of mixed somatic nerves with all the different vegetative and muscular components. It is known that infusion of epinephrine and norepinephrine causes fall in the intraosseous pressure, and stimulation of the peripheral end of the divided splanchnic nerve has an identical effect. (Shim et al. 1972).

Central haemodynamic parameters also exceed influence upon bone perfusion measured as intraosseous pressure. Herzig and Root (1959) and Weiss and Root (1959) showed that occlusion of the femoral artery causes fall in the intraosseous pressure. Stimulation of the sciatic nerve in dogs causes immediate increase in intraosseous pressure, possibly by increasing venous outflow resistance. (Shim et al. 1972).

These findings imply that nerves are present in bones and we have to believe that increased sympathetic activity reduces bone blood flow, and that decreased central arterial blood pressure exceeds a similar effect.

The total arterial inflow in bone gets its contributaries from three main supplies. Cuthberthson et al. (1964) found after ligature of the nutrient artery a transient fall in intraosseous pressure. 50 per cent of the bones studied were able to restore their pressure within 3 hours. This indicates that bone on the macrocirculatory level at least is a functional unit. This is most pronounced in mature bone as the epiphyseal plate is a vascular barrier in immaturity.

Brookes (1957) in his study of the importance of the epiphyseometaphyseal vessels, found after destruction of the nutrient channel that this hardly affected the longitudinal growth in long bones in rabbits. A shortening of 3 per cent on the operated side showed that the extremities of tubular bone are provided with sufficient blood supply from the vessels penetrating the cortex near the epiphyseal plate.

These studies indicate that the long bone has to be considered as a multiple inlet/multiple outlet system and interference with one of the main contributaries of blood might change the haemodynamic in bone. However, in respect to the growth processes, bone receives sufficient nutrition after destruction of one of the main supplies. It therefore seems logical to believe that these contributaries might take over the nutritional function if one of them is destroyed.

Infusion of vasoactive drugs and hormones causes differences in bone perfusion. However, these experiments causes variations in the central arterial blood pressure or in the regional vasomotorical tonus in the limbs studied, and redistribution or steal phenomenon might invalidate the conclusions made.

All these experiments are performed on intact anaesthetized animals, but are not quite conclusive, as selective stimulation of the vasomotoric nerves to the bone at present is impossible. Stimulation of mixed nerves causes stimulation of all components and stimulates all areas in the supplying regions of this nerve. Steal phenomenon and redistribution of the blood in a limb make conclusions difficult.

It seems, however, as if the sympathetic nerves tend to decrease the bone blood flow, while physiological stimuli to obtain vasodilatation in bone are unknown.

# Chapter II

## INTRAMEDULLARY PRESSURE, ITS NATURE AND PHYSIOLOGICAL SIGNIFICANCE

Since Rothman (1913) measured the medullary pressure it has been known that all bones have a positive pressure in their interior. Larsen (1938) measured intramedullary pressure as one of the first and detected that bone underwent necrosis after high pressure infusion of saline. Stein & al. (1957) measured variations in normal bone marrow pressure in anaesthetized dogs and found a positive pressure with considerable variation and with differences in different regions in tubular bone. However, their animals were not mature and it is know that immaturity increases bone perfusion (Whiteside & al. 1977). Shim & al. (1972) suggested a positive correlation between intramedullary pressure and bone blood flow. The measured, however, flow as outflow in the nutrient vein. This might be dubious as we have to consider bone as a multiple inlet/multiple outlet system. Shaw (1963) measured bone blood flow with coupled thermocouples and found a positive correlation between medullary pressure and bone blood flow. Azuma (1964) found in rabbits, great variations in intramedullary pressure and he also detected differences in epiphyseal versus metaphyseal pressure. He also performed microscopy on the measuring site in the bone and found that the cannula had been immersed in a blood pool and that small arteries, venoules and sinusoides had contributed to the net positive pressure. He also found a correlation between inflow to the bone and medullary pressure.

Michelsen (1967) measured medullary pressure in rabbit tibias and compared this to the pressure in a small emissary vein outside the bone. He found equal values of medullary pressure compared to venous pressure outside the bone. He could not reproduce regional differences found by Shim & al. (1972) and Azuma (1964). Furthermore, he detected that medullary pressures are even in an area and that the intramedullary pressure and the venous pressure in that region are of the same magnitude. Marrow pressure was determined in different parts of the bone marrow cavity by invasive technique, but not linked to anatomical location. Wilkes & Vissher (1975) measured medullary pressure in dogs with an atraumatic technique. They were able to remove the cortical bone without destroying the endosteal membrane. The pressure found was not different from pressure measured with invasive technique.

In humans Arnoldi & al. (1972) and Arlet & al. (1968) have measured intramedullary pressure during normal and osteoarthrotic conditions. The normal intramedullary pressure was in the same range as pressure measured in aminals but with smaller variations. In osteoarthritis the medullary pressure was increased without an increase in femoral vein pressure (Arnoldi & al., 1972). This is contrary to the observations of Michelsen (1967) but it is evident that pathological conditions in the bones might increase venous outflow resistance. Many investigations (Stein & al., 1958, Herzig & Root, 1959, Michelsen, 1967) have shown a decreasing effect of adrenaline and sympathetic nerve stimulation on the medullary pressure.

Electric stimulation of the sciatic nerve in the dog causes decrease in

tibial medullary pressure (Shim & al., 1972). All this implies that the relation between medullary pressure and cancellous bone blood flow exists, and that the sympathetic stimulation decreases medullary pressure by causing vasoconstriction in the arterial system. Furthermore it is commonly accepted that a decrease in arterial blood pressure causes decrease in medullary pressure, and that the increase in peripheral vascular resistance is due to vasoconstriction of the arterioles.

This also means that the medullary pressure is subject to alterations and as a continous measurable parameter reflects the circulatory conditions in bone. However, the absolute magnitude seems to be of less value when considering perfusion of diaphyseal bone (Polster, 1970). However, relative measurements may reflect the haemodynamic conditions in long bones (Held & Thron, 1962, Shaw, 1963, Azuma, 1964).

# Chapter III

## TECHNIQUE WHEN MEASURING MEDULLARY PRESSURE

To obtain reproducible values the following technique is recommended to avoid technical errors.

The cannula should have an internal diameter of at least 1.6 mm and of a strength that allows the penetration of cortical bone without damage to the cannula. A burr could be used to penetrate the cortex but extensive surgery to expose the cortex should be avoided as this will impair the arterial blood supply to the bone. A sharp cannula able to penetrate the cortex could be used when applying sufficient force on the superficial part of the cannula. X-ray imaging is very useful to confirm the exact position of the tip of the needle. Once introduced in place the cannula should remain unmanipulated and by no circumstances withdrawn. This will affect the magnitude of the blood pool around the tip of the cannula. The superficial part of the cannula should be connected to pressure sensitive registration equipment by means rigid plastic tubing. Preferably straingauge transducer and amplifier could be used. It is valuable to record the appearance of the medullary curve as only pulsatile curves reflect the medullary pressure. Between the transducer and cannula a device allowing very small amounts of fluid to enter the tubing and connected cannula should be equipped as this very slow flow in the tubes with heparinized saline will secure against clotting at the tip of the cannula. The Interflow® system used in this experiment allows 50 microliter saline to flush the tube every minute and this does not affect neither the pressure measured nor the pressure in the bone.

# Chapter IV

## THE INFLUENCE OF THE ARTERIAL BLOOD PRESSURE ON THE MEDULLARY PRESSURE IN TUBULAR BONES IN DOGS

All modern workers dealing with medullary pressure use the equipment previously described. The medullary pressure shows fluctuations which are pulse synchronous. A typical recording is shown below from one of our experiments.

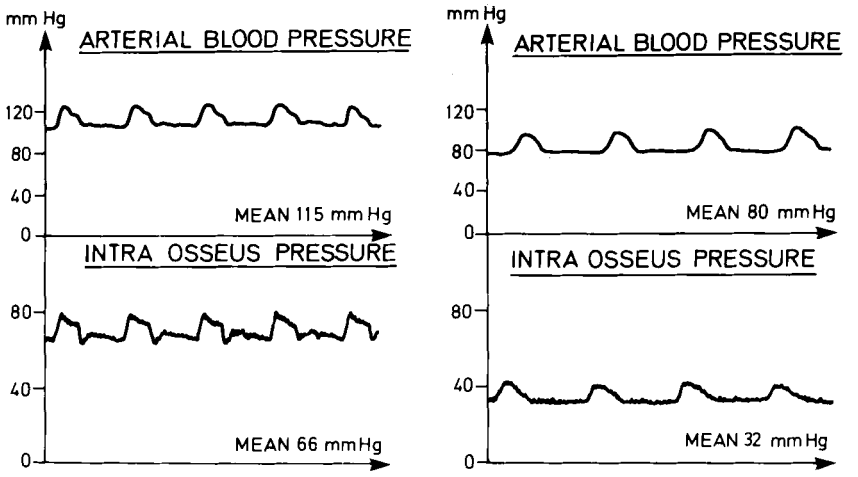


*Figure 4.1*
*The dependence of the intraosseous and arterial pressure is shown. At left a recording with an arterial blood pressure of 115 mm Hg and the corresponding recording from intraosseous measuring site. Below a recording from the same measuring site after reduction of the arterial blood pressure to 80 mm Hg.*

The pressure measured in bone shows nearly arterial configuration. When decreasing the arterial blood pressure in the anaesthetized animal by reducing the cardiac output (measured with Swan-Ganz thermodilution technique) and maintaining blood gases and peripheral vascular resistance constant (within 10 per cent), the medullary pressure decreases suddenly when the mean arterial blood pressure reaches a level around 80 mm Hg.

According to Stainsby (1973) most organs and tissues in the body are able to control blood flow in proportion to need for nutrition. Autoregulation of blood flow means ability of organs to maintain a constant blood flow in face of changes in arterial perfusion pressure. The classical organs with a well established autoregulation of blood flow are the brain and the kidneys.

Autoregulation seems to be present in bone as well. According to figure 4.2.a.b.c. shown below it is evident that the medullary pressure is constant at all measuring sites if the arterial pressure is kept above 80 mm Hg. It should be mentioned that during the experiments on which this conclusion is based, all arterial blood gases are kept constant and all tissues are at rest with suggested minimal metabolic needs. When decreasing arterial blood pressure

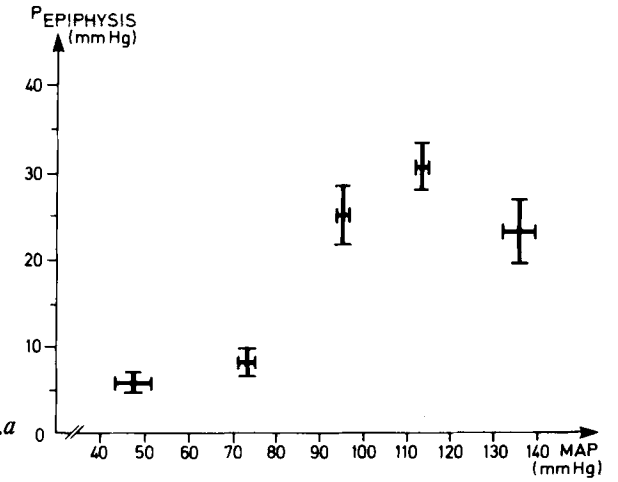


*Figure 4.2.a*

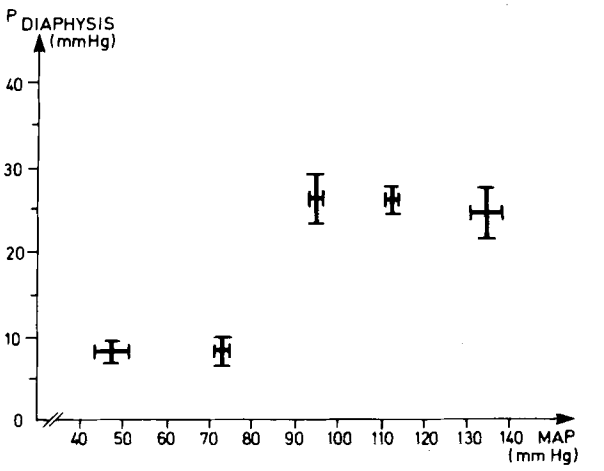


*Figure 4.2.b*

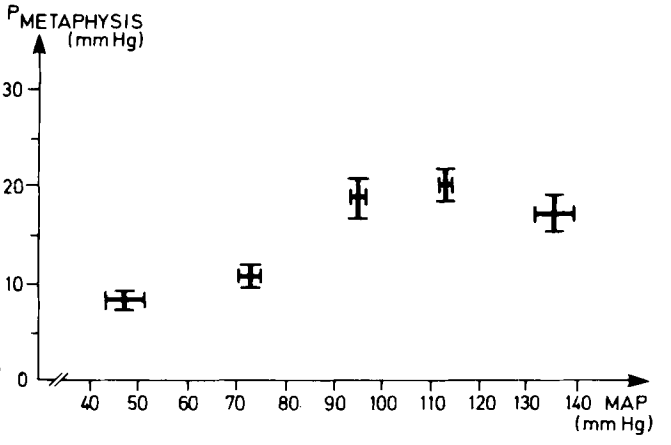


*Figure 4.2.c*

*Figure 4.2.a.b.c*
*The dependency between the intraosseous and arterial blood pressure is shown in figure 4.2. There is no difference in pressure in the different locations in the tubular bones. Note the significant pressure fall when the arterial pressure (MAP) is below 80 mm Hg. For further details: Tøndevold et al. (1979 a).*

below 80 mm Hg a significant reduction in medullary pressure takes place. As earlier mentioned this as a relative measure means a decrease of arterial inflow in bone. The pressure reduction in the bone is very sudden and according to figure 4.1 the pulse height decreases whereas the pressure declines sharply. This very sharp fall takes place within 5–10 mm Hg variation around this critical value in mean arterial pressure. It should be mentioned that the central venous pressure and pulmonal arterial pressure were constant during this reduction of cardiac output.

The physiological significance of this "threshold" phenomenon means that when blood pressure falls the bones are capable of maintaining constant perfusion above 80 mm Hg in mean arterial pressure. Below this level the autoregulation of blood flow breaks and the cardiac output is directed to more vital organs in the body like brain, kidneys and heart. This threshold is known to be lower in the vital organs (Strandgaard & al., 1975, Rothe & al., 1971 and Haunsø, 1981). According to Shaw (1963) and Shim & al. (1972) the infusion of adrenaline causes decrease in medullary pressure. However, their doses might be considered unphysiological as the whole central circulation is altered considerably. An increased sympathetic activity could by increasing the vascular resistance in the medullary area thus divert blood to vital organs. This vasomotion in bone seems suitable in the overall regulation of the circulation.

## THE INFLUENCE OF THE ARTERIAL OXYGEN TENSION ON THE MEDULLARY PRESSURE

Oxygen is essential for the normal metabolism in all tissues. Oxygen exceeds great influence upon the haemodynamic condition in the main circulation as well as it does in the local control of the capillary perfusion. Hypoxia is a powerful stimulus of the sympathetic nervous system. This means that vasoconstriction is mediated via sympathetic nerves to all tissues of no immediate importance during hypoxaemia (Folkow & al., 1961).

The oxygen tension participation in the local control of blood flow during hypoxia is more a matter of dispute. There are evidences that oxygen acts directly on the vascular smooth muscles and causes dilatation during hypoxaemia or by other transmitters (Carrier & al., 1964, Detar & Bohr, 1968). There are evidences that hypoxaemia through a direct effect affects the vascular resistance in an organ. Local alterations in tissue metabolism might take place during hypoxaemia, and this altered metabolism might dilate the resistance vessels (Johnson, 1974).

When looking at figure 4.3 it is clearly shown that a reduced arterial oxygen tension below 75 mm Hg causes reduction in bone perfusion, due either to an increased sympathetic tone and/or steal phenomenon from other tissue with a higher ability of vasomotion than bone.

Ross & al. (1962) have demonstrated that decreasing arterial oxygen tension in the blood to the lower limb in dogs causes an increase in blood flow of the femoral artery. This means that a vasodilatation takes place during hypoxaemia in the tissues and there are evidences that oxygen or absence of it acts as a stimulus for this physiological event.

The question of a blood flow reduction in the bone due to a steal pheno-

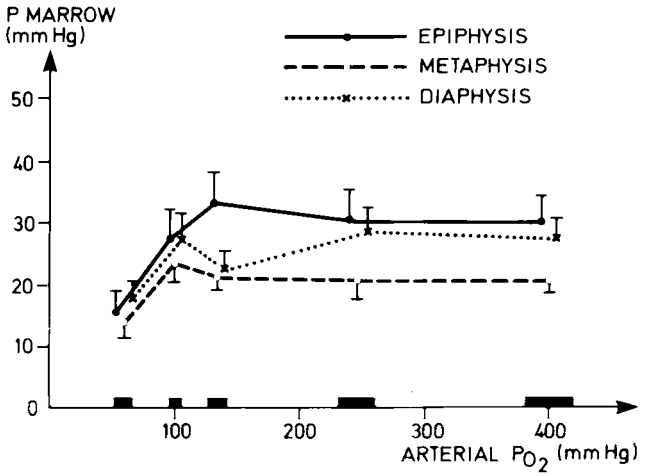


*Figure 4.3*
*The relation between the intraosseous pressure and arterial oxygen tension. The intraosseous pressure is independent of arterial oxygen tension as long as the tension is above 75 mm Hg.*

menon is unanswered. It seems probable that vasodilatation in the surrounding tissue decreases the effective inflow pressure to the medullary area. This means shunting of the blood to more vital organs in the overall distribution. It is known that hypoxaemia is a powerful stimulator of the sympathetic nervous system. The increased sympathetic tonus might explain the phenomenon by direction of blood to muscular tissue on behalf of bone perfusion.

When regarding the intact hind limb of an animal it is obvious that most of the tissue is muscular tissue. Skin, fat, bones and other tissue represent a smaller fraction of the total weight. The skeletal muscles have the ability to increase their perfusion about twenty five times during exercise (Paaske and Sejrsen, 1980). It is unknown to what extent the bone is able to perform functional or reactive hyperaemia. In a limb it seems more probable that during a high level of sympathetic tone or during postocclusional hyperaemia, the bone is among the tissues which are less able to change their perfusion. The reduced resistance or increased conductance in the muscular vessel might cause a steal phenomenon. This will reduce bone perfusion while muscular perfusion is regarded more essential in the overall distribution.

## THE RELATION BETWEEN MEDULLARY BLOOD GAS COMPOSITION AND ARTERIAL BLOOD GASES

It is obvious that the red marrow consumes oxygen and produces $CO_2$ in its metabolism. All tissues in the body have an oxygen demand. Bone shows pressure induced autoregulation. It is known that active osteogenesis is dependent on a certain $CO_2$ tension and is increased by a raised $CO_2$ tension (Wilmer, 1965). Paff (1948) found that in tissue cultures a low pH mean increased mineral deposition and bone formation. Goldhaber (1958) has demonstrated that oxygen favours bone removal in tissue cultures.

All this indicates that the medullary gas composition in some way is linked to osteogenesis. In living mammals, Brookes (1966) has demonstrated

that the venous engorgement after femoral vein ligation produces raised $CO_2$ tension and a low pH below the ligature. In pathological conditions like osteoarthritis of the hip Arnoldi & al. (1972) have demonstrated venous engorgement and again a connection between disturbed gas composition in the bone and increased bone formation is suggested.

Semb (1966) demonstrates that immobilisation and disuse osteoporosis induces a raised pH in the medullary blood from rabbit tibias. Microscopically an increase in bone vascularity and an increased number of resorption cavities were demonstrated in cortical bone.

In the present work by Eriksen & al. (1979) we could demonstrate a linear relation between arterial blood gas tension and medullary gas tension in a range which covered the physiological area. The studies performed on the medullary gases during stable conditions show rather similar results except for oxygen.

$CO_2$ which plays an active part in the control of pH and also osteogenesis showed a linear connection between arterial $CO_2$ tension and medullary $CO_2$ tension.

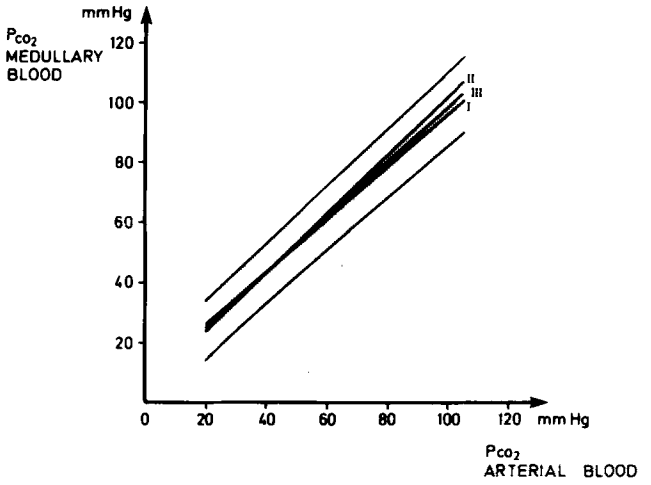


*Figure 4.4*
*The relation between arterial $CO_2$ tension and $CO_2$ tension in the intraosseous circulation.*

This indicates that the production of $CO_2$ in the bone is small in regard to the perfusion during resting conditions. It is unknown to what extent the medulla has capabilities to work anaerobically, but Brookes & Helal (1968) have found a medullary $CO_2$ tension of 77 mm Hg in an impacted fracture of the femoral neck.

Kiivisaari & Niinkoski (1975) found increased $CO_2$ tension in healing bone in rabbits, and could also by selective blockade of the carbon-dioxide producing system with acetazolamide (carbon anhydrase inhibitor) show that it is possible to increase bone $CO_2$ tension without interference with arterial tension. This shows local production of $CO_2$ during increased metabolic conditions during fracture repair.

Contrary to the arterial blood tension of oxygen, $CO_2$ variation in the arterial blood did not induce changes in medullary pressure.

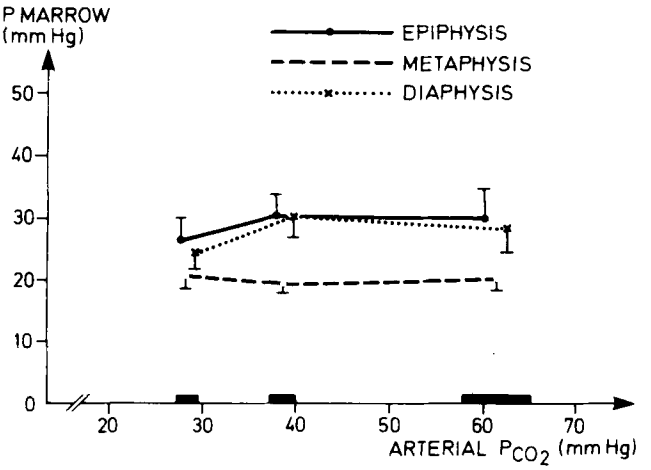


*Figure 4.5*
*The correlation between intraosseous pressure and arterial $CO_2$ tension.*

When changing the arterial carbon dioxide tension within the physiological range, no difference in medullary pressure in neither diaphyseal, epiphyseal nor metaphyseal bone was found. This means that carbon dioxide content in bone seems correlated to the arterial $CO_2$ tension and that no active regulation exists. This is in spite of the osteogenic effect of carbon dioxide.

Poyart & al. (1975) have studied the distribution of $CO_2$ in bone and found 60–70 per cent in the form of carbonate in bone crystals. The rest is more or less in a more easily exchangeable pool. They found the exchange of $CO_2$ flow limited, and this corresponds well with the finding that $CO_2$ is more a question of perfusion than active ability of bone to regulate the tension of this osteogenic important gas. It is also interesting to note that Duling (1973) found an increase of 70 per cent in tissue oxygen tension when restoring the tissue carbon dioxide tension. This means that the correct oxygenation of the tissues demands a certain level of $CO_2$.

## THE RELATION BETWEEN ARTERIAL pH AND STANDARD BICARBONATE COMPARED WITH THE CORRESPONDING MEDULLARY VALUES

According to Post & Shoemaker (1962) infusion of acid to anaesthetized animals causes decrease in outflow of venous blood from the femoral bones in dogs. Surprisingly the opposite takes place when loading unanaesthetized aminals with acid. When changing the arterial composition of $CO_2$, pH and standard bicarbonate a similar difference took place in the blood gases obtained from the medullary cannulas.

The values detected in medullary blood of local pH and standard bicarbonate seem directly correlated to arterial values, indicating that bones do not contain buffer systems within our range of measurements.

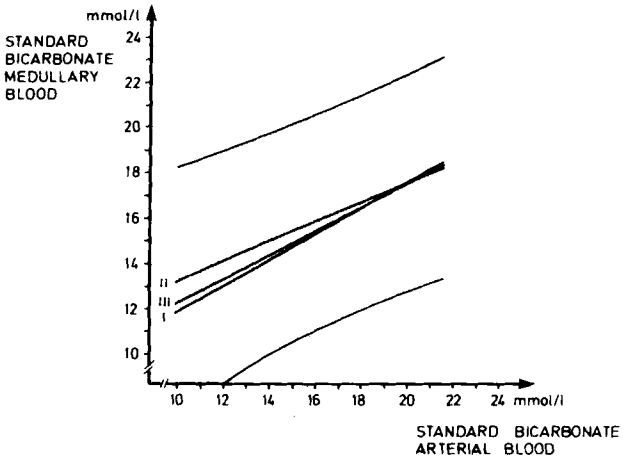


*Figure 4.6*
*The relation between intraosseous standard bicarbonate and corresponding arterial value. 95% confidence limits are shown.*

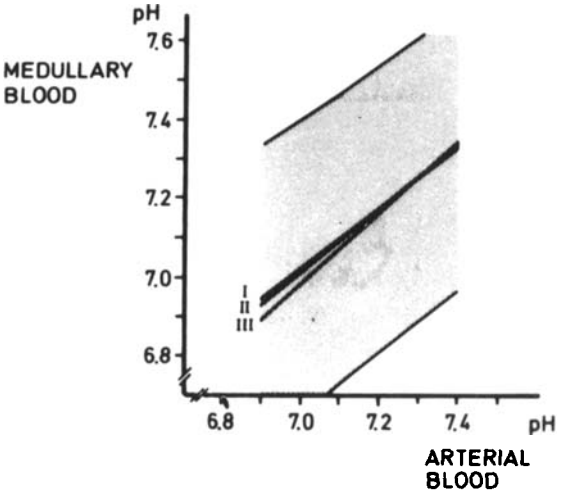


*Figure 4.7*
*The relation between arterial pH and intraosseous pH. 95% confidence limits shown.*

## THE INFLUENCE OF ARTERIAL pH UPON THE MEDULLARY PRESSURE

In the present experiments the arterial pH did not exert any influence upon the medullary pressure. This is shown in figure 4.8.

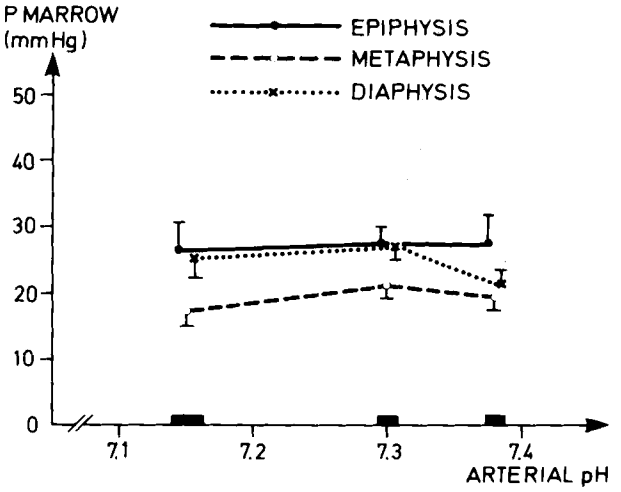


*Figure 4.8*
*The dependency between intraosseous pressure and arterial pH.*

# Chapter V

## THE MEASUREMENT OF PERFUSION IN DIAPHYSEAL BONES

### Earlier Methods

In the literature great differences exist concerning the blood flow rate in bone. As mentioned, bones consist of different tissues with different functions. The supporting function of the diaphysis is combined with erythropoietic functions in the bone ends and to some extent in the medulla of even mature bones. The ability of the individual longitudinal growth is closely related to the epiphysis.

The role of intramedullary pressure measurements in determination of the vascular conditions has been treated, and shall not be repeated here, but the aim is to deserve other techniques some attention. Cumming (1962) measured bone perfusion recording venous outflow from the nutrient vein after ligation of all other tributaries. As bone has to be considered as a multiple inlet/multiple outlet system, this method might be invalidated by fractional collection of the venous output.

Shaw (1964) measured bone blood flow by means of heated thermocouple technique. The method is however invasive and the amount of reactive hyperaemia at the measuring site after the violent introduction of the measuring probe is subjected to differences in interpreting the results of the measurements performed. In 1955 Frederickson & al. used $^{45}Ca$ clearance as a measurement of bone blood flow. Later Semb (1966), Bosch (1969) and Shim & al. (1971) used different bone seeking isotopes in blood flow determinations. The Fick principle has been quite natural to use as it is a well established method in physiological blood flow studies. Schoutens & al. (1979) have shown by this method that the amount of isotopes which can be extracted from the blood is limited and the extraction rate for $^{45}Ca$ is 60 per cent when the blood flow is low and 25 per cent when blood flow increases. They therefore concluded that initial bone clearance is inadequate to monitor differences in blood flow rates when these exceeds a certain magnitude.

Other techniques include $^{125}I$-iodoantipyrin washout described by Kelly (1971), the hydrogen washout introduced by Whiteside & al. (1977) and the $^{133}Xe$ washout method described by Lathinen & al. (1979). The principles in these methods are the introduction of a radioactive isotope and the external registration of the declining activity over the measuring site, according to the washout of this isotope by the circulating blood. These methods are invasive and require some surgery on the extremity upon which the measurements are destinated to take place. Tissue inhomogenicity and differences in affinity of the tracer for different parts of the tissue also make the results obtained difficult to interpret.

The technical problems seem to have been solved by Whiteside & al. (1977). The possibility to perform experiments on unanaesthetized individuals seems practically impossible by these techniques.

Niv & al. (1980) have demonstrated an identity when using this hydrogen wash-out technique compared with microsphere technique in the femoral heads in dogs.

## FLOW MEASUREMENTS WITH THE USE OF RADIOLABELLED MICROSPHERES

The microsphere technique is based on the use of spherical corpuscle of different size and material usually labelled with a gamma emitting isotope. Microspheres are widely used in blood flow studies concerning inaccessible organs or tissues, as the reference sample technique makes it possible to compare activity in tissue versus that in arterial blood. Before using microspheres in estimation of blood flow the following criterias have to be fulfilled:

1) the microspheres have to be evenly suspended in the blood,
2) the microspheres have to be of a size which assures embolisation during first passage in the circulation,
3) the total number of microspheres injected must be of such a quantity that the amount in each biopsy taken exceeds a certain number due to the need for statistical treatment of the results,
4) the microspheres have to be of uniform size and charge. The isotope must not be liberated and removed from the spheres after embolisation in the tissue.

In practical ways to obtain reproducible data concerning blood flow measurements in bone the following has to be remembered:

1) a selection of a suitable size of microspheres, 15 μ, is preferable. There is evidence that larger spheres are located in the axial stream of the larger vessels (Phibbs & Dong, 1970).
2) it is also preferable to use reference sampling technique. This means that a pump or an artificial organ with known constant perfusion is placed in parallel with the organ with unknown perfusion rate. The perfusion rate in the organ could thus be calculated from the formula,

$$\text{blood flow rate} = \frac{\text{activity/100 g tissue} \cdot \text{ref. sample blood flow}}{\text{activity in reference sample}}$$

3) injection of sufficient number is necessary. According to Buckberg & al. (1971) at least 383 spheres have to be trapped in the biopsy to obtain reproducibility at a 95 per cent level,
4) selection of radiolabelling which permits counting with least effort is preferable, that means in most practical way counting of gamma emitting isotopes with an energy that minimizes attenuation through the bones.

It is a matter of dispute if the microspheres have to be injected in the left atrium or it is sufficient to inject the material in the left ventricle. In our studies of the perfusion rate in bones we have obtained adequate mixing after injection in the left ventricle expressed as activity in different parts of the circulation during the injection procedure. The differences in activities in samples obtained from different parts of the arterial circulation turn out to be negligeable or within 0.1 per cent. This simplifies the procedure as this can be achieved by the aid of a pig tail catheter introduced into the left ventricle and therefore does not require thoracotomy (Figure 5.1).

The microspheres used in some of the experiments were made of human serum albumine. The values obtained with these spheres were not different from those determined with carbonized microspheres.

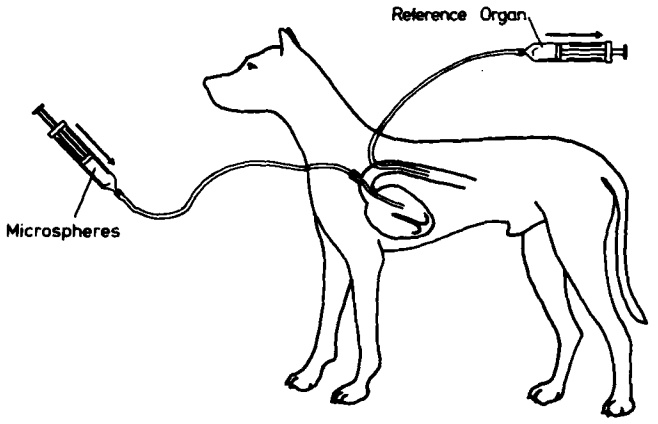


*Figure 5.1*
*The experimental animal with catheters in position. Note reference sample pump.*

Kaihara & al. (1968) found the left atrium as the best injection site, while Hales (1974) and Sasaki & Wagner (1971) obtained sufficient reproducibility and mixing of the spheres after intraventricular injection.

There is further indication that the axial accumulation of spheres in the central stream of the arterial vessels makes the method less reproducible. Aukland (1980) doubts if the method is suitable in the kidney due to skimming of the spheres. In bone this is an unsolved question at present, but it is shown (Tøndevold & Eliasen, 1982 b) that the small vessel haematocrit in the bones is approximately 50–75 per cent of the arterial haematocrit, and it might be possible that a similar phenomenon takes place concerning the distribution of the microspheres in bone. However, the differences in regional perfusion in long bones are of a magnitude that makes this answer more an acaedemic question that a question of practic measurement.

Another question which has to be answered is to what extent the microspheres affect the microcirculation in the tissue after the spheres have been trapped in the small vessel. According to Hales (1974) he has not been able to register any physiological significance after sphere injection upon all cardiovascular parameters. It should also be mentioned that glomerular filtration rate in the kidneys is unaffected by injection of spheres for blood flow measurements (Hales, 1974).

## LIMITATIONS IN THE MICROSPHERE TECHNIQUE

The microsphere and the reference sampling technique are only measuring blood flow in a biopsy in a certain moment. The results obtained are thus some sort of a momentary value of the perfusion rate. To obtain more prolonged duration of the measurements the microspheres might be infused. This has, however, not been done in the present experiments. This also means that the microsphere technique is a discontinuous way of measuring the flow rate. Repeated injections make it possible to follow variations in perfusion rate during dfferent situations, but the microspheres used on that occasion have to be labelled with different isotopes. Another limitation is

the invasive character of the method. It is necessary to inject in the arterial part of the circulation, to collect arterial blood and tissue samples, and finally determine the amount of radioactivity in the biopsies and the blood reference sample. This restricts the use of microspheres at present to use in animals, but refinement in techniques and preparation might allow their use in humans. Axial accumulation of large spheres makes also flow assessments doubtful (Aukland, 1980).

## REGIONAL MEASUREMENTS OF BONE PERFUSION IN CONSCIOUS AND ANAESTHETIZED DOGS

As pointed out the microsphere method compares activities in different regions with acitivities in tissues with known perfusion. This means that the flow obtained is a momentary picture of the circulatory situation. The blood flow value obtained is the blood flow rate at that moment. Repeated measurements require different labelling of the microspheres. To obtain reproducible measurements in anaesthetized animals it is essential that the circulatory conditions permit entrance of blood to bone. As earlier mentioned hypoxaemia, hypotension and sympathetic stimulation decrease blood flow to bones. Our findings (Tøndevold & al. 1979, a,b) obtained with medullary pressure measurements are confirmed by Gross & al. (1979) using microspheres. During haemorrage, adenosine vasodilation and hypoxaemia they found increased vascular resistance. During normal physiological conditions in conscious dogs this is of minor importance, but in the artifical situation on the experimental table it is crucial that the circulatory condition is satisfactory to permit blood to enter the interior of the long bones.

A sufficient number of microspheres injected and a stable circulation permit measurements in minor areas in all tissues of the body. It is possible to perform studies on conscious animals and even in humans, as the microspheres do not seem to affect the organism neither generally nor locally. Brookes (1965) found in rats a variable content of red cells in the different

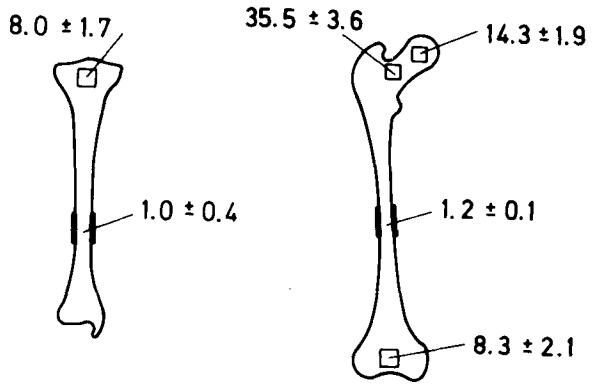


*Figure 5.2*
*The perfusion rates in different areas of long bones measured with $^{99}Tc^{m}$-labelled human albumin microspheres.*

parts of the rat femoral bone. This indicates regional differences in the vascular volume. The pattern with a low cortical blood flow compared with a more rapid rate in cancellous bone is outlined in this work and the phenomenon is clearly demonstrated in as well awake as anaesthetized animals in experiments by Tøndevold & Eliasen (1982, a, b) and Tøndevold & Bülow (1983). The values determined when performing regional perfusion measurements are shown in table 5.1 and the distinct location of biopsies are shown in figure 5.2.

*Table 5.1*
*Perfusion rates in different anatomical regions in the hind limb bones in resting conscious and anaesthetized dogs.*
*All flow rates in ml · (100 g tissue)$^{-1}$ · min$^{-1}$ ± SEM. N = 12.*

| | |
|---|---|
| Femoral head | |
| Anaesthetized dogs | 14.4 ± 1.9 |
| Awake dogs | 12.6 ± 1.1 |
| Femoral neck | |
| Anaesthetized dogs | 35.5 ± 3.6 |
| Awake dogs | 27.3 ± 3.1 |
| Cortical bone | |
| Anaesthetized dogs | 1.2 ± 0.1 |
| Awake dogs | 1.6 ± 0.2 |
| Supracondylar cancellous bone | |
| Anaesthetized dogs | 8.3 ± 1.7 |
| Awake dogs | 6.3 ± 0.8 |
| Tibial condylar bone | |
| Anaesthetized dogs | 8.0 ± 1.7 |
| Awake dogs | 2.6 ± 0.5 |

All authors dealing with blood flow measurements in bone have determined differences in mixed cortical and mixed cancellous bone (Lunde & Michelsen, 1970, Morris & Kelly, 1980, Okubo & al., 1979). However, long bones have to be considered as an organ with different functions and perfusion rates according to metabolic needs. The overall systemic regulation of peripheral vascular tone also affects bone blood flow rates. Different functional areas in bone seem to be responsible for the differences in perfusion rates. It is therefore essential in comparative studies on different individuals that the biopsies are taken from equally reproducible areas. The perfusion rates in the femoral neck are 30 times the values determined in cortical bone. Any erythropoietic tissue mixed in the cortical biopsies will greatly affect the blood flow rates determined.

In measurements obtained from femoral supracondylar and tibial condylar bone this phenomenon is most clearly demonstrated. The amount of fat

in this region seems related to the age of the animal. In the first series of experiments (Tøndevold & Eliasen, 1982, a) the perfusion rates in these regions were measured to 27 and 29 ml · (100 g tissue)$^{-1}$ · min$^{-1}$. The animals used in this series were by occasion relatively young, with mostly red marrow in these regions. In a later study the mongrel dogs used were older and the perfusion thus determined in equal locations was found to be 8.3 and 8.0 ml · (100 g tissue)$^{-1}$ · min$^{-1}$.

Adipose tissue at "rest" has a perfusion rate ranging from 2.8 ml · (100 g tissue)$^{-1}$ · min$^{-1}$ in a popliteal fat depot to around 10 ml · (100 g tissue)$^{-1}$ · min$^{-1}$ in subcutaneous tissue (Bülow & Tøndevold, 1982). Variable amounts of adipose tissue in bone therefore may affect the perfusion rate measured.

## BONE BLOOD FLOW DURING MODERATE HEAVY EXERCISE

Drinker & Drinker et al. (1922) demonstrated that stimulation of the nerve accompanying the nutrient artery caused vasoconstriction in isolated specimens from the dog's tibia. Valderrama and Trueta (1965) found that stimulation of the sciatic nerve in the anaesthetized animals increased the intraosseous pressure, and that this effect could be blocked with tubocurarine chloride in dogs. They interpreted this rise as secondary to increased outlet resistance and occlusion of the venous outflow.

Muscle contraction thus seems to affect the outflow of blood from the long bones. Gross & al. (1979), however, performed experiments on conscious dogs during moderate exercise. Using microspheres as blood flow indicator they state that during exercise vasoconstriction takes place. The conclusion might be dubious, as the blood flow determined is equal during rest and exercise periods, but the increased vascular resistance might be a result of differences in the net pressure gradient in the vascular system. During muscular work the blood pressure increases. Valderrama & Trueta (1965) demonstrated increased intraosseous pressure and venous pressure in the limb following stimulation of the sciatic nerve. If the venous pressure in the small collecting vessels in bone increases during muscular activity by the same magnitude as the arterial blood pressure increases, then the vascular resistance remains constant.

In the present study an influence upon bone perfusion at muscular exercise was detected. A rise in blood flow rate takes place after about one hour of moderate exercise has elapsed. After two hours submaximal exercise upon the treadmill, with an accompanying rise in plasma lactate, the perfusion rate in areas with low perfusion increased more than 50 per cent. (Table 5.2).

This delayed hyperaemia was observed after a period of one hour after the start of the muscular exercise, but interestingly the increase was sustained at least 45 minutes after the cessation of the exercise period. This is presumably an expression of a repayment phenomenon conditioned of some metabolic deficits. The delayed hyperaemic response could be demonstrated in cortical bone from both tibia and femur, and also in supracondylar femoral bones, and tibial condylar bones.

In the femoral neck the perfusion at rest was relatively high (27.3 ml · (100 g tissue)$^{-1}$ · min$^{-1}$), and no statistical increase in blood flow could be

found during exercise. It seems as if this area has a relatively high blood flow rate during resting conditions presumably to assure sufficient nutrition during muscular activity. In the femoral head an increase from 12.6 to 20.6 ml · (100 g tissue)$^{-1}$ · min$^{-1}$ was determined.

Thus the duration of exercise might be an important factor, when functional hyperaemia is measured in long bones. In regions with a heavy load during exercise blood flow seems to stay high or it will increase after one to two hours. In cortical bone the perfusion in the second hour rose from 1.6 to 2.5 ml · (100 g tissue)$^{-1}$ · min$^{-1}$.

*Table 5.2*

| Prework | 1 hour | 2 hour | After work |
|---|---|---|---|
| 12.6 ± 1.1 | 13.7 ± 1.5 | 20.1 ± 3.2* | 17.7 ± 2.0 |
| 27.3 ± 3.1 | 23.2 ± 2.6 | 36.9 ± 6.2 | 34.8 ± 3.4 |
| 1.6 ± 0.2 | 2.1 ± 0.2 | 2.5 ± 0.1* | 2.6 ± 0.3* |
| 6.3 ± 0.8 | 7.2 ± 0.7 | 10.5 ± 2.3* | 11.2 ± 1.6* |
| 2.6 ± 0.5 | 3.6 ± 1.0* | 3.7 ± 0.8* | 3.5 ± 0.7* |
| 1.6 ± 0.2 | 2.1 ± 0.2 | 2.5 ± 0.2* | 2.6 ± 0.3* |

*The flows in ml · (100 g tissue)$^{-1}$ · min$^{-1}$ for different regions in long bones in the hind limb are listed. The mean and standard error of the mean are given. Values different from the preexercise, resting values are marked with an asterisk. **

In the supracondylar femoral region the flow increased from 6.3 to 10.5 ml · (100 g tissue)$^{-1}$ · min$^{-1}$, and the tibial condylar plateau showed a similar increase in the second hour from 2.6 to 3.7 ml · (100 g tissue)$^{-1}$ · min$^{-1}$.

It should be mentioned that all measurements in bones is subject to a reasonable variation. There are great interindividual variations which might hide a physiologic effect. The two ways variance test and corresponding t-tests used secures significance at 95 per cent level, and eliminates the biologic variation, but not the physiologically induced effect (Armitage, 1974).

It is generally accepted that in skeletal muscle the flow increase which occurs during exercise is due to factors inside the muscle itself. This vasodilatation is unaffected by sympathectomy or by chronic somatic denevation. (Sparkes, 1978). The mechanisms responsible for this hyperaemia in bone are at present unknown, but we feel that if neural elements should be involved, the flow response should be faster than measured in this experiment. (Tøndevold & Bülow, 1983).

# Chapter VI

## REGIONAL BLOOD VOLUMES IN BONE

### Introduction

The existence of differences in regional perfusion has been discussed in the preceeding chapters. Furthermore the vascular anatomy of bone is in part sinuisodal. Further questions of importance in the understanding of the circulation in bone are as follows:

1. Do the different "vascular" indicators penetrate the walls of the exchange vessel to the same extent, at the same speed, or do they act as ideal vascular indicators?
2. Do the functional blood capacity and the perfusion correlate to each other, or do areas exist with small vessel capacity, and high perfusion rate?
3. Does there exist any difference in the proportion between erythrocytes and blood plasma in different parts or is the blood flowing through the vessels with the same composition as in the arterial part of the circulation?

The following will try to make an approximation to the answers of these questions.

## DETERMINATIONS OF ERYTHROCYTE VOLUME IN BONE

The haematocrit in the interior of bone until present has been unknown, it has not been possible to determine blood volume simply by determining one of the components of the blood, and correct by the arterial haematocrit found in the peripheral circulation. As mentioned later the haematocrit in the bones depends on the location of measurement, but it is in the range 50–75 per cent of the arterial values.

Performing measurements of as well erythrocyte volume, plasma volume and perfusion rate the connection could be established. Brookes (1965) has determined the volumes in the rat femoral bones of immature animals. His finding of 1.874 ml erythrocytes per 100 g tissue in red marrow in the inferior metaphysis in the rat femur is in fact not different from the present finding of 1.39 ml erythrocytes per 100 g tissue in the red cancellous bone of the dog's femoral neck. However, his technique is impossible to use in larger animals and thus in the study of regional differences and physiological variations.

The canine erythrocyte is difficult to handle. In the present study (Tøndevold & Eliasen, 1982 b) the erythrocytes haemolyzed during most of the conventional methods used in the labelling procedure. It was therefore necessary to find a method which could assure the incorporation of $^{51}Cr$ in the erythrocyte without altering the rheological properties of the individual corpuscle. This method is listed in detail in appendix 1. It should also be mentioned that splenectomia has to be performed, as the spleen in the dog is capable of altering the arterial haematocrit in the intact animal from 40–50 per cent, but not in the splenectomized dog (Dietz & al., 1979).

## THE DISTRIBUTION VOLUMES OF PLASMA INDICATORS IN BONE

The determinations of tissue haematocrit necessitated an evaluation of the different plasma indicators used in the volume measurements. The most widely used plasma indicator is serum albumin labelled with a radioactive isotope. It is known that albumin passes to the extravascular space to some extent, but the exact overestimation when using this indicator in bone is according to my knowledge unknown.

Bell & al. (1980) found differences in extravascular distribution volumes of albumin and fibrinogen in the dog hind paw. Watson & al. (1980) found a lymph/plasma concentration of 0.24 for albumin and 0.04 for fibrinogen. The lymph used was collected from mixed tissue in the hind paw in dogs. This can be interpreted as a relatively more pronounced extravasation of albumin. Bone does not contain lymphatic channels (Seligher, 1970; Brookes, 1971) and the extravasation of the albumin might therefore be different from other tissues. The work of Owen and Trifitt (1976) has revealed that only 16 per cent of the albumin in bone is intravascular. The albumin is used as a protein in the biosynthesis of bone. The extravasation seems to invalidate the use of albumin as a plasma volume indicator in most tissues in the body, when using techniques similar to the technique used in the present study (Tøndevold & Eliasen, 1982 b).

Hosain & al. (1969) have outlined a method of plasma volume determination using $^{113}In^{m}$ bound to plasma transferrin. The technique is very simple, and an advantage is that the labelling may be done in vivo.

*Table 6.1*
*The diffusion coefficient and molecular weight of the indicators used. All diffusion coefficients are expressed as $cm^2 \cdot sec^{-1}$ and all molecular weights are in $g \cdot mol^{-1}$.*

| Indicator | Diff. coeff. | Mol. weight | % Impurities |
|---|---|---|---|
| $^{125}$I-Fibrinogen | $8.5 \times 10^{-8}$ | 340.000 | 5% |
| $^{113}In^{m}$-Transferrin | $5.3 \times 10^{-7}$ | 57.000 | 50% |
| $^{99}Tc^{m}$-Albumin | $3.4 \times 10^{-7}$ | 69.000 | 5% |

Different physical constants for the indicators reveal that albumin is an ellipsoid molecule with a Stokes-Einstein radius of 36 Å. The molecular weight of albumin is approximately 69.000 g/mol, compared to the molecular weight of 340.000 g/mol for fibrinogen. The fibrinogen molecule is rod-shaped with a Stokes-Einstein radius of approximately 108 Å and with a dimension of 700 · 38 Å. Physical datas for the $^{113}In^{m}$-transferrin complex is of no interest, as it is evident in the study that in our hands it was impossible to get the $^{113}In^{m}$-isotope to bind with the transferrin-molecule. It seems from our studies that the $^{113}In^{m}$-isotope labels albumin and not the high molecular transferrin (molecular weight approximately 400.000 $g \cdot mol^{-1}$). Due to technical problems further exploring in the exact localisation of $^{113}In^{m}$ was not performed.

The diffusion coefficient was determined in agar gel for $^{99}Tc^{m}$-albumin, $^{113}$In-transferrin and $^{125}$I-fibrinogen. The results show that $^{113}In^{m}$-trans-

ferrin and $^{99}Tc^{m}$-albumin had nearly equal properties, but low molecular impurities in the $^{113}In^{m}$-transferrin were considerable, but this does not seem to exert any measurable effect in vivo presumably due to binding of $^{113}In^{m}$ to other molecules with high molecular weight (Table 6.1).

The three indicators were injected in heparinized dogs and the plasma concentration and plasma disappearance curves were followed. The time from injection of the isotope to sacrifice was 10, 30 and 60 minutes. All plasma disappearance curves followed the same pattern. This is shown in figure 6.1 below. The start concentration in the plasma for albumin was always much higher than the equilibrium or final concentration, but it declines sharply and is in levels with the others after about 5–6 minutes. This indicates that the egress of albumin takes place in the first minutes after the aggregate has been injected. This is in accordance with results found by Sejrsen & al. (1982) showing that after an intraarterial bolus injection of $^{131}I$-albumin a considerable extravasation of the albumin took place during a normal passage through the vascular system in skeletal muscle tissue (Henriksen et al., 1982).

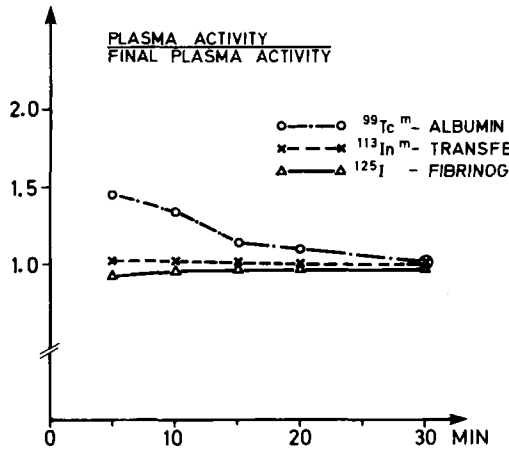


*Figure 6.1*
*Concentration of vascular indicator in blood plasma at different time intervals.*

In the present experiments the following procedure was used.

After the death of the aminals the bones were excised, frozen and all biopsies were taken with a saw from the earlier outlined regions.

The distribution volumes determined for the different indicators in the tissue are not equal. According to table 6.2 the distribution in the tissue of $^{113}In^{m}$-transferrin and $^{99}Tc^{m}$-albumin were about the same. $^{125}I$-fibrinogen however showed a smaller distribution volume or about half the volume determined with the other indicators. In brain tissue, however, which was selected as a biological control organ, the blood brain barrier excluded the smaller indicators from permeation of the capillary wall. The volume determined in the brain with the three indicators are remarkbly equal. This demonstrates that the two indicators with lowest molecular weight yields plasma volumes in bone tissue about twice the value obtained with the indicator having the large molecular weight. This means that the haematocrit determined from

the estimated erythrocyte and plasma volumes will be half the value found with the use of estimates for the large molecular indicator. This finding is in accordance with results obtained by Bell & al. (1980) who determined a larger extravascular distribution space for albumin than for fibrinogen.

The 5 per cent molecular impurities found in fibrinogen did not exert any measurable effect upon the values found for plasma volumes in bone. After repeated washings of the fibrinogen and reduction of impurities from 5 per cent to 5 per thousand, no measurable effect could be found upon the distribution measurements. The techniques used are outlined by Sejrsen (1976). All this invalidates albumin as a plasma volume indicator as the equilibration across the exchange vessels takes place within the first 10 to 20 minutes. The fibrinogen used seems favourable, but it requires the addition of heparin to the animal or human used. It was found that the low energy (35 KeV) of $^{125}I$ did not cause any attenuation through the bone pieces in the counting tube (Table 6.2).

*Table 6.2*
*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 $(100g)^{-1}$ ± l.s.d. | $^{125}I$-Fibrinogen ml $(100g)^{-1}$ ± l.s.d. | $^{113}In^{m}$-Transferrin ml $(100g)^{-1}$ ± l.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 |

## BLOOD VOLUME AND FLOW DETERMINATIONS IN DIFFERENT REGIONS IN LONG BONES

The perfusion rates in the biopsies which were taken at different places according to the earlier mentioned functional different regions, were measured with the microsphere method and reference sampling technique. Blood volume in a biopsy is the sum obtained from the separate determination of erythrocyte and plasma volume. The different isotopes were counted in a three channel system and differentiated according to the different levels of their photopeak. The amount of gamma energy was thus found in every biopsy and the acitivity of every isotope per gram tissue could be determined.

According to table 6.3 the relations between blood flow and blood volume are shown. It is of importance to notice that the increase in vascular volumes found in the different parts are followed by an equal increase in perfusion rates. The blood volumes found in cortical bone are 25 times less than the corresponding volumes determined in the cancellous red marrow contain-

ing areas in the femoral neck, and the same effect is shown for the perfusion rates in the mentioned locations.

The other regions are in an intermediate position due to varying amounts of bone and fat tissue in the biopsies.

*Table 6.3*
*The distribution volumes of $^{125}$I-fibrinogen as plasma indicator and $^{51}$Cr-labelled 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 supra-condylar 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 |

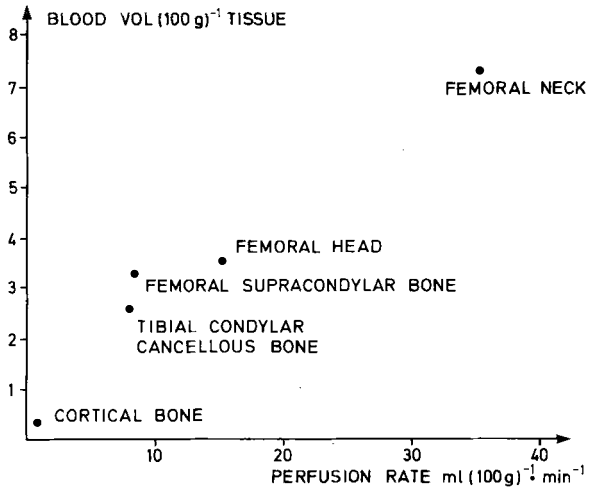


*Figure 6.2*
*Data from table 6.3 shown graphically. Increases in blood volumes are connected with a corresponding increase in blood flow.*

The figure 6.2 illustrates more graphically this phenomenon. Even if the differences on the figure do not mean that the perfusion rate and blood volume could be altered to the same extent in a topographical location, it is assumed that the vascular volumes determined in the bones are due to an increased perfusion rate. This makes it necessary to recall the technical procedure used. Meanwhile the $^{125}$I-fibrinogen and $^{51}$Cr-labelled erythrocytes are circulating the microspheres are injected and the perfusion rate this detected. While all haemodynamic parameters including blood gases are stable, the dogs are killed with a saturated dose of KCl. This means asystoli in diastole. Rapid excision of the bones with as little blood loss as possible and rapid freezing then secured as optimal conditions as possible. It is of course an open question to what extent the blood volumes in the small vessels in bone are altered when the circulation stops. However, in the first minutes after death the venous pressure in the central circulation is above zero, and after dissection of the bones the inferior caval vein is tense upon palpation. It seems that this way of sacrificing the animals simply takes the arterial pressure off in a few seconds and maintains the venous backpressure. Therefore the suspected outflow of blood from the bones during the agony seems less likely, but it is impossible to detect this source of error with the technical possibilities available at present. Another point which has to be discussed is, to what extent the presence of microspheres in the capillaries and small arterioles reduces the determined vascular volume. According to Hales (1974) only 0.01 per cent of the capillaries are blocked and the error seems negligeable.

It seems according to this logical to conclude that blood volume and perfusion rate are correlated in a linear way in bone tissue. This means that when talking about the functional circulatory conditions in bones, the different perfusion rates found correspond to the estimated blood volumes. The capacity of the vessels seems at rest in the supporting cortical bone to be equal and very small compared with the sinusoideal vessels of the red marrow and the cancellous bone.

## STUDIES OF THE HAEMATOCRIT IN DIFFERENT REGIONS IN BONE

In 1929 Fahraeus reported that if the non-Newtonian fluid blood, flowed through tubes with small diameters the haematocrit of the blood in the tube decreased when the tubediameter decreased. The reason for this phenomenon seems to be axial accumulation and faster flowing axial stream of the corpuscular elements in the small vessels. Haematocrits have been determined in different tissues in the body in the small vessels and the capillaries.

Jodal & Lundgreen (1970) determined the haematocrits in the intestinal tract in cats using $^{125}$I-labelled human serum albumin as plasma indicator and $^{51}$Cr-labelled erythrocytes. Their finding of a tissue haematocrit of 50 per cent of the arterial value in the mucosa of the small intestine correlates well with Johnson & al. (1971) and Klitzman and Duling (1979). The latter found a haematocrit in the cremaster muscle in hamsters around 26 per cent of the arterial value. Their technique however, is different from other authors' as they are looking directly upon the small vessels, not only measuring net values in a tissue biopsy. During maximal vasodilatation they found a rise in haematocrit to 75 per cent of the arterial value.

The results obtained with different methods are somewhat conflicting as Jodal & Lundgreen (1970), when reducing mucosal blood flow, determine higher haematocrit in the intestinal mucosa, and at vasodilatation they found no significant effect upon tissue haematocrit.

In the present study no attempt was undertaken to alter the blood flow rate in bone. The blood flow rates are similar to values obtained from conscious dogs (Tøndevold & Bülow, 1983).

The blood flow rates determined by Tøndevold & Eliasen (1982 b) are values obtained during resting conditions. The haematocrits detected are well below the arterial values. The tissue haematocrit detected by Jodal & Lundgreen (1970) and in the present study are a mixture of the haematocrit values in all the small vessels in the biopsy. Smaller changes cannot be determined using this technique, but is unfortunately the only possible at present in bones.

Johnson & al. (1971) found variations in the capillary haematocrit during spontaneous vasomotion with a decrease during stimulation of the sympathetic nerves (Krogh, 1919) and during hyperoxia (Klitzman & Duling, 1979). Increasing haematocrit was detected during functional hyperaemia (Klitzman & Duling, 1979). To what extent low capillary haematocrit can be a result of shunting of the corpuscular elements or is subjected to regulation according to tissue oxygen delivery demand is a question to be answered in the future.

Brookes (1971) has tried to determine the haematocrit in bone. After collection of blood from the nutrient vein with a haematocrit of 60 per cent, he calculates the cortical haematocrit to 42 per cent. The reason for having this haemoconcentration in the nutrient vein is difficult to explain as lymphatic channels are absent in bone. An increase in haematocrit from arterial value 33 per cent to nutrient vein 60 per cent is not understandable.

The present tissue haematocrit determinations are made after separate measurements of plasma and erythrocyte volume in the different regions in bone. As later mentioned the use of plasma indicators is a matter of debate. All volume determinations performed are dependant on the biological validity of this parameter.

In table 6.3 the different values determined for plasma volumes and erythrocyte volumes in bone are listed. It is shown that haematocrit values in this normalized table are well below the arterial haematocrit values of 40 per cent. In the femoral head a haematocrit of 20 per cent or 50 per cent of the arterial value was calculated. In the femoral neck where the perfusion rate was 2.5 times larger, the haematocrit was about the same, 19 per cent. In cancellous bone from the distal femoral epiphysis the haematocrit was 22 per cent, and on the other side of the knee in the tibial plateau the haematocrit was 21 per cent. In cortical bone from femur and tibia the perfusion rates were low, 1.2 and 1.0 ml · (100 g tissue)$^{-1}$ · min$^{-1}$, but the haematocrit was 30 and 33 per cent respectively. This means that the low tissue haematocrit present in other tissues in the body is found to be present in bone as well. The differences in haematocrit and perfusion rates are difficult to order in a specific pattern, but it is evident that the slowly perfused areas have higher haematocrit values than regions with higher perfusion rates. This also is a confirmation of work done by Brånemark (1959) who found a higher erythrocyte velocity in the cortex than in the marrow by vital microscopi. If

the individual small vessel in cortical and cancellous bone constitute the same relative volume, the erythrocyte velocity in the cortex will be larger according to the higher haematocrit. In Fahraeus' (1929) work, however, the higher haematocrit should imply larger vessels, but this could not be confirmed. The field is at present somewhat confusing and the answers are ahead in the future.

# General conclusion

Concerning the circulation in long bones in anaesthetized and conscious dogs the following has been demonstrated in the present study:

1) The long bones have the ability to regulate the inflow of blood from the central circulation in spite of variations in as well arterial blood pressure as arterial oxygen tension. Bone has to be considered as an organ with autoregulation of blood flow. Using medullary pressure measurements as a continuous registration of a parameter correlated to blood flow rates, it was shown that the arterial inflow stops when the arterial blood pressure falls below 80 mm Hg and the arterial oxygen tension below 75 mm Hg.
2) There exists great difference in the blood perfusion rates in different regions in the long bones. The perfusion rates measured using microspheres are 1–3 ml · (100 g tissue)$^{-1}$ · min$^{-1}$ in the cortical bone compared with a perfusion rate of 35–50 ml · (100 g tissue)$^{-1}$ · min$^{-1}$ in the cancellous bone in the femoral neck. The perfusion in the different regions depends possibly on the mechanical load and the relative amount of haematopoetic tissue in the region.
3) During physical exercise the long bones increased their perfusion with 50–75 per cent of the resting value, and the hyperaemia is maintained nearly 1 hour after termination of the exercise period.
4) There exists a linear correlation between blood volume and regional blood perfusion rate in bone tissue.
5) The dynamic haematocrit in different regions in bone is 50–75 per cent of the arterial haematocrit. The lowest haematocrits are found in regions with the highest perfusion rates.
6) There exists a considerable extravascular albumin space in all parts of bone as the plasma volume determined with the macromolecular indicator $^{125}$I-fibrinogen (molecular weight 340.000 g · mol$^{-1}$) is about half the value measured with albumin (molecular weight 69.000 g · mol$^{-1}$) as plasma indicator.

# Appendix 1

**$^{51}$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. 40 ml of arterial blood (anticoagulated with citrate) was centrifuged by 300 G in 15 minutes. The plasma was removed and the buffy coat discarded. 600 µCi $Na_2$ $^{51}CrO_4$ (Radiochemical Center, Amersham) was added and incubated for 30 minutes at 37°C. The erythrocyte suspension was washed twice in its own plasma and centrifuged by 300 G in 15 minutes between the washings. The final suspension contained microscopically normal erythrocytes. After removal of the spleen there was no disappearance of $^{51}$Cr activity in two 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 erythrycyte 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).

# Summary in Danish

Arbejdets formål er at belyse den lange rørknogles cirkulationsfysiologiske grundparametre. Der stilles følgende spørgsmål, som med de tekniske målemetoders reservationer, søges besvaret i det følgende:

1) Har rørknoglen autoregulation og hvad er grænserne herfor?
2) Er blodgasserne i det intraossøse miljø parallelt foranderlige med de centrale blodgassers værdier?
3) Er der forskel i regional perfusion i knoglen eller er gennemblødningen ens i en rørknogles forskellige, anatomiske partier?
4) Kan rørknoglens perfusionshastigheder forandres ved fysiologiske stimuli?
5) Er der nogen sammenhæng mellem gennemblødningen og blodvolumen regionalt, eller er der områder med sinusoidal karakter med stort blodvolumen og lav perfusion?
6) Eksisterer der nogen væsentlig interstitiel væskefase i knogler eller er ekstravaskulærfasen negligeabel?
7) Har knoglen identisk fordelingsvolumen for de anvendte plasmaindikatorer, eller er der en molekylevægtsbetinget forskel i fordelingen?

KAPITEL I

Rørknoglens cirkulationsanatomi gennemgås, og ud fra litteraturen opstilles den arterielle forskydning som kommende fra følgende tilløb:

1) Arteria nutricia.
2) Metafysære og epifysære arterier.
3) Periostale arterioler fra periost samt muskel- og senefæster.

Efter at karrene har gennemløbet et transcorticalt forløb deler arterierne sig i ascenderende og descenderende grene. Herefter nås til sinusoidale væv, hvorefter veneafløbet sker dels i småvener og dels i en stor, central sinus. De inderste 2/3 af cortex ernæres fra kar i marvkanalen. Udvekslingskarrene er formodentlig af kontinuerlig, ikke fenestreret type.

Derefter gennemgås det kendskab man har til knoglens cirkulationsfysiologiske mekanisme. Der er nervevæv i knoglen, og man forestiller sig, at såvel sympaticusstimulation som infusion af adrenalin og noradrenalin, medfører en vasokonstriktion, enten i knoglen eller uden for knoglen. Knoglen må anses for at være et organ der reagerer som en funktionel vaskulær enhed, og er af typen multiple inlet/multiple outlet system.

KAPITEL II

Det intraossøse tryks karakter gennemgås og tidligere tolkninger af dette tryk vurderes. Validiteten af det interossøse tryk som et flow parameter må være i situationer hvor relative målinger kan foretages.

KAPITEL III

De tekniske forudsætninger for at måle det intraossøse tryk gennemgås detaljeret.

KAPITEL IV

Forfatterens egne forsøg med intraossøse trykmålinger på bedøvede hunde gennemgås. Der postuleres med intraossøs trykmåling, at knoglen har en tryk-induceret autoregulationsmekanisme, idet det intraossøse tryk er konstant med arterielt blodtryk over 80 mm Hg, men under denne værdi optræder en tærskel med et hurtigt, intraossøst trykfald.

Hypoxæmi medfører en identisk reaktion, idet en arteriel ilttension under 75 mm Hg medfører udshunting af knoglen.

Herved placeres knoglen lavt i den generelle kredsløbsregulation, idet trykfald og hypoxæmi medfører nedsættelse af knoglens perfusion, eller en redistribution af blodet, afhængig af organernes vitale funktioner.

I øvrigt er der ingen indflydelse på knogleperfusionen ved forandringer i $pCO_2$ eller pH i arterieblodet.

I øvrigt følger det intraossøse miljø stort set gasvariationerne i det centralt cirkulerende blod.

KAPITEL V

Der redegøres for de tidligere historiske metoder til kvantitativt at fastlægge knoglens perfusionskoefficient. Den af forfatteren anvendte metode, microsphæreteknikken, gennemgås, og forudsætninger samt metodens begrænsninger fastlægges i detaljer. Der redegøres for forfatterens eksperimentelle påvisning af betydelige, regionale perfusionsforskelle, hvor blandt andet gennemblødning i cortical knogle er 30 gange mindre end i hæmopoetisk aktiv rød marv. Det fundne gennemblødningsmønster er identisk på vågne og anæstheserede dyr. Under muskelarbejde påvises en tardiv funktionel hyperæmi i knoglen, idet man efter 2 timers kontinuerligt løb på et løbebånd, får en 75% forøgelse af gennemblødningen i knoglens dårligst perfunderede afsnit.

KAPITEL VI

For at bestemme den mikrovaskulære hæmatokrit i knoglebiopsier, er de 2 forskellige blodfaser gennemgået. Vor metode til $^{51}Cr$-mærkning af erythrocytter er gennemgået. Derefter gennemgås fordelingsvolumina for forskellige plasmaindikatorer. Det er således at man efter kun få minutters ekvilibreringstid, får et dobbelt så stort volumen for albumin som for fibrinogen. Der påvises derefter en direkte sammenhæng mellem perfusion og blodvolumen. Den mikrovaskulære eller småkars hæmatokrit bestemmes, og findes til ca. halvdelen af den centrale, arterielle hæmatokrit, som er normaliseret i forsøget til 40%.

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