STUDIES OF BONE BLOOD FLOW DISTURBANCES AFTER FRACTURE

OF THE ADULT TIBIAL DIAPHYSIS

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Union of fractures of the adult tibial shaft is often delayed after high energy accidents, and this may result from greater devascularisation of the bone in these injuries. The aim of this study was to develop a radioactive microsphere technique for the measurement of bone blood flow in the adult rabbit in order to investigate quantitatively the routes of bone blood supply after fracture and the effects of fracturing force on blood flow after blunt trauma.

After demonstrating that two isotope labels could be reliably separated in the tissue samples, the optimum size of microsphere and the number of microspheres allowing two measurements of flow in each animal were tested. This technique was employed to show that unilateral immobilisation of the hindlimb in a cast did not result in any differences between the limbs in tibial flow after one or two weeks.

After unilateral surgical osteotomies of the tibial diaphysis immobilised in a cast, cortical flow increased but that to the marrow did not, indicating that the flow response in the cortex is mediated by a supply parallel to that to the marrow. The cortical blood flow changes were abolished by the exclusion of the periosteum and soft tissue by a sheath, suggesting that this parallel supply is principally from these tissues. The cortical flow was not significantly reduced by one or two weeks after exclusion of the marrow circulation by an intramedullary nail.

Fractures of the tibial shaft were produced by percussion. The blood flow changes after lower and higher energy injuries were not significantly different, and were similar to those found after a surgical osteotomy although at one week the marrow flow was depressed. No evidence was therefore found in this study that higher energy fractures produced by blunt trauma are associated with a depressed blood flow response.

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PART 2   VALIDATION OF ANIMAL MODEL

PART 3   EXPERIMENTAL TIBIAL FRACTURES

PREFACE

The time to union of fractures of the adult tibial shaft is commonly prolonged, and this has long been attributed to vascular factors. However, until the development of the radioactive microsphere technique, the measurement of bone blood flow has presented problems, and a non-invasive method for use in humans has yet to be perfected. As a result, the quantitative information available on the changes in blood flow after tibial diaphyseal fracture is limited in scope and reliability. In the work presented in the following pages, an animal model for the measurement of tibial blood flow by the microsphere technique has been developed, and this method applied to quantify bone flow after experimental fractures. This has enabled more detailed study of the flows to the different parts of the diaphysis, and the rôles of the main routes of blood supply. Further, the method has been extended for the first time to the study of fractures caused by blunt trauma.

The project was completed with the assistance of a number of members of this and other departments.

First and foremost I am indebted to Prof PJ Gregg, Professor and Head of the Department of Orthopaedic Surgery, University of Leicester, for giving me the opportunity of joining the Department to undertake this work. He has supervised the project at every stage, and assisted in the drafting of this manuscript. His support has made the project possible.

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Hilary Stafford, Chief Technician of the Department of Orthopaedic Surgery, undertook the ordering of the microspheres and other consumables, and advised on the use of laboratory equipment. Sue Gardner and Sujatta Dutt, of the University Department of Medicine, acted as Radiation Protection Supervisors and assisted with the loan of equipment. Dr Bob Bing, Senior Lecturer in Medicine, kindly consented to act as Departmental Radiation Protection Officer, and permitted the use of the departmental gamma counter.

John Baker, University Radiation Protection Officer, gave unstinting help in the difficult task of running the project within the limits of the isotope disposal capacity. Steve Bruce, of the Biomedical Services Unit, overlooked the disposal of carcasses within these limits.

Dr John Thompson, Lecturer in Epidemiology of the Department of Ophthalmology, gave valuable advice on the statistics used throughout the work.

Colin Morrison, Principal Experimental Officer of the University Department of Engineering, drew up the plans for

the fracture rig, and supervised its manufacture by the departmental workshop. Dr Dick Stephen, Senior Lecturer in Clinical Physiology, advised on the assessment of fracturing energy *in vivo*.

The microsaw used for osteotomy was loaned by the Zimmer company, and the casting materials were donated by Johnson and Johnson.

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PART 1

INTRODUCTION

1.1

FRACTURES OF THE ADULT TIBIAL DIAPHYSIS

1.1.1 FRACTURE UNION IN THE TIBIAL DIAPHYSIS

The best treatment for closed fractures of the adult tibial shaft remains controversial. While most such fractures managed either conservatively or by operation proceed to union, others heal very slowly or apparently not at all. The reasons for this are not known, and this makes the choice of treatment for an individual fracture the more uncertain.

While no clinical series can give the 'natural history' of healing, inasmuch as all the fractures considered have been treated, those fractures managed by closed methods give the most useful information about those factors affecting time to union that depend principally on the nature of the injury rather than on the nature of the treatment. However, defining the end point of fracture healing is not without difficulty even in the absence of the internal splintage provided by operative fixation.

In clinical practice, union is usually defined as having occurred if the fracture site is painless, with no tenderness or instability on manual examination, and with bony trabeculae crossing the fracture site on radiological examination. While the shaft of the tibia is among the easiest of long bones to test manually, as it is subcutaneous throughout its length, manual examination may not detect small movements at the fracture site, and it is not practicable to examine the patient every day. The assessment of union is thus performed at intervals, often of several weeks, rather than continuously. Radiologically, reliable conclusions on the presence or otherwise of bony

trabeculae crossing the fracture site can be made only if the fracture is in one plane and the X-ray beam is in that plane, a combination which virtually never occurs in clinical practice. Radiological healing often lags behind clinical union, and a fracture may remain very obvious on a radiograph despite restoration of function to the limb. Techniques devised to give an objective assessment of union, such as vibration transmission and stress testing (Sekiguchi and Hirayama 1979, Nokes *et al.* 1985, Hammer 1985), are yet to be widely accepted and available, or to be applied to a large detailed series of fractures.

In studies of closed management, union is commonly defined as having occurred when the patient is allowed full weightbearing without splintage. With this definition, the average time to union of closed fractures of the adult tibial diaphysis is of the order of sixteen to twenty weeks (Nicoll 1964, Johnson and Pope 1977, Austin 1981, Digby *et al.* 1983, Sarmiento *et al.* 1984), but the distribution about this mean is skewed. Up to 14% of fractures take over twenty six weeks to heal, and some take over a year (Nicoll 1964, Sarmiento *et al.* 1984). This *delayed union* represents considerable morbidity.

1.1.2 CLINICAL STUDIES OF TIBIAL DIAPHYSEAL FRACTURE UNION

The factors affecting the incidence of delayed union may be considered in the light of the results of studies performed over the past thirty years (Ellis 1958, Nicoll 1964, Johnson and Pope 1977, Austin 1981, Digby *et al.* 1983, Keller 1983, Sarmiento *et al.* 1984, Oni, Hui and Gregg 1988).

Fracture pattern

The pattern of a tibial shaft fracture reflects the mechanism of injury (Johner and Wruhs 1983). Three basic types are generally recognised (Figure 1.1.1 a-c):

1. *Transverse* - sustained by a direct blow without axial loading.

2. *Oblique* - sustained by a direct blow during axial loading. The magnitude of the angle of the fracture to the long axis of the shaft that differentiates an oblique from a transverse fracture is arbitrary and often not stated, but is frequently taken as 45° (Bauer and Edwards 1965, Edwards 1965a, Johnson and Pope 1977).

3. *Spiral* - sustained by a twisting force about the long axis. This type is differentiated from an oblique fracture by the presence of a longitudinal limb linking the upper and lower extents of the fracture (Johnson and Pope 1977, Johner and Wruhs 1983).

All three types may be comminuted, *ie*, comprise more than two fragments, and this occurs in at least two circumstances:

(a) when the fracture is caused by more than one force acting in different directions. For example, an oblique fracture may have a 'butterfly' fragment if a bending force is combined with the axial loading (Figure 1.1.1 d).

(b) when higher forces are applied over a greater area. A special example of this is a segmental fracture or 'bumper' double fracture caused by a relatively wide object such as a car bumper (Figure 1.1.1 e).

In some cases, a portion of the shaft may actually be lost.

The proportions of the various types in each study group depends on the exposure of the population studied to different causes of tibial shaft fracture. Different workers have also grouped fracture types differently. However, the fracture pattern *per se* has not been found to be correlated with delayed union, but rather the presence or degree of comminution of the fracture (Ellis 1958, Nicoll 1964, Johnson and Pope 1977, Digby *et al*. 1983, Oni, Hui and Gregg 1988).

Fracture level

The position of the fracture along the tibia is usually assigned to the upper, middle or lower third, accounting for 3 to 16%, 28 to 55%, and 36 to 58% of fractures respectively (Nicoll 1964, Johner and Wruhs 1983, Oni, Hui and Gregg 1988). There is no generally agreed method of assigning a fracture that crosses the dividing line between thirds. Further, segmental and extensively comminuted fractures do not readily fit into such a classification, but these have often been treated operatively and therefore not included in the studies in question.

While it is generally believed that fractures of the distal third heal more slowly, clinical studies indicate that the location of the fracture has no independent effect on the rate of union (Ellis 1958, Nicoll 1964, Johnson and Pope 1977, Sarmiento *et al*. 1984).

Fracture displacement

The distal fragment of a shaft fracture may be angled, shifted or rotated with respect to the proximal fragment, and if shifted by at least the width of the bone at the fracture site it may override the proximal fragment so as to result in shortening. However, in clinical studies, shift alone has generally been considered, no doubt because it is easier to quantify from a radiograph than rotation, and significant shortening can occur only with concurrent shift. All types of displacement are likely to be underestimated because of the partial reduction resulting from first aid measures taken before the radiographs are obtained. It is thus not surprising that in general only the more severe degrees of displacement have been found to have a consistent relationship to the time to fracture union, having an association with prolonged healing time (Ellis 1958, Nicoll 1964, Johnson and Pope 1977, Austin 1981, Digby *et al*. 1983).

Associated fibular fracture

It is commonly believed that an intact fibula will slow healing by 'jacking' apart the tibial fracture surfaces. In these clinical studies, this factor has been considered without controlling for other factors, and it has been found that the opposite is the case, *ie*, a fractured fibula is associated with longer times to union. This effect is probably dependent on the concurrently increased displacement of the tibial fracture (Nicoll 1964, Digby *et al*. 1983, Sarmiento *et al*. 1984, Oni, Hui and Gregg 1988).

Soft tissue injury

Soft tissue injury associated with a shaft fracture varies from a minor contusion to major loss of skin and muscle combined with neurovascular injury. Grading systems that have been developed to quantify these injuries generally include compound fractures only, as no reliable method yet exists to assess clinically the soft tissue involvement in closed fractures (Oestern and Tscherne 1984). Compounding is found to be associated with delayed union, particularly if severe (Ellis 1958, Nicoll 1964, Digby *et al*. 1983, Sarmiento *et al*. 1984).

These studies have shown that the factors associated with slowed healing include fracture comminution, initial displacement, associated fibular fracture, and compounding. These features are in turn related to the degree of force causing the fracture (Hulth 1980), and it has been shown that, as a group, closed fractures sustained in higher energy accidents have a higher incidence of the delay of union beyond twenty weeks than lower energy injuries when treated by closed methods (Oni, Hui and Gregg 1988). This effect may be related to a higher level of injury to the blood supply of the bone and its surrounding tissues.

1.1.3 TIBIAL DIAPHYSEAL BLOOD SUPPLY AND FRACTURE UNION

It is commonly believed that a good blood supply is a
necessary requirement for the healing of fractures (Brookes
et al. 1970, Trueta 1974, Rhinelander 1974, Gustilo *et al.*
1990). The high rate of delayed union of the tibial shaft
in comparison with that of the femur is in turn said to
result from the relatively poor coverage of the bone by
vascular muscular tissue, particularly over its distal
third (Trueta 1974), and the greater degree of vascular
derangement expected in fractures caused by higher energy
impacts may constitute the principal mechanism of delayed
union. Comminuted fractures will have one or more bony
fragments that are partially or completely denuded of soft
tissue and marrow and thus of their blood supply, while the
more severely displaced fractures will have a complete
interruption of the medullary circulation and will be
subject to a variable degree of muscular and periosteal
stripping, with or without the avulsion of the nutrient
vessels entering the shaft. The degree of devascularisation
is probably greatest in severe compound fractures
associated with loss of soft tissue, and these are the
fractures that are most prone to delayed union.

Richards and Schemitsch (1989) studied the importance
of the soft tissue by creating a devascularised segment of
the canine tibia by removing and replacing a length of the
diaphysis between two osteotomies. After one month, a
higher proportion of the osteotomies were united when the
segment was covered by a muscle flap rather than by the
original skin, and this was thought to be related to the
greater blood flow found in muscle flaps (Fisher and Wood
1987). Similarly, union is delayed by temporary devascular-
isation of the muscle surrounding an excised and replaced
segment of the rabbit radius (Holden 1972), by exclusion of
the periosteum and soft tissue around an osteotomy of the
rabbit radius or tibia by a sheath (Cavadias and Trueta
1965, Oni 1987), and by simple separation of the muscle

from the periosteum at the site of an osteotomy of the dog radius (Kolodny 1923).

This effect is especially marked if the nutrient vessels are also destroyed (Kolodny 1923, Cavadias and Trueta 1965, Whiteside and Lesker 1978b, Oni 1987). Haw *et al.* (1978) studied upper diaphyseal segments of the canine tibia replaced in their beds and internally fixed with a plate. Those segments with their nutrient vessels reanastomosed united earlier than those left devascularised. However, this difference was probably principally related to the difference in the rate of infection, which reached 75% in the devascularised segments. Teissier *et al.* (1985), in a similar study of the rabbit fibula, found that on average the revascularised shafts united one week earlier, a relatively long period of time in an animal whose long bone fractures normally heal in four to six weeks.

Union after clinical fracture often occurs normally despite severe ischaemia of the neighbouring muscles (Owen and Tsimboukis 1967). This ischaemia results from rises of pressure within the osteofascial compartments of the leg after fracture, causing the closure of veins and small arterial vessels and constituting a *compartment syndrome* (Rorabeck and Macnab 1976, Moore and Cardea 1977, Halpern and Nagel 1980, Allen *et al.* 1985, Gershuni *et al.* 1987). Such syndromes might depress tibial blood flow in the short term by occlusion of osseous venous drainage, and in the longer term by the inhibition of the revascularisation of the fragments by subsequent fibrosis of the muscle. Court-Brown and McQueen (1987) claim that delay in union follows such syndromes, but their study was retrospective and dependent on the diagnosis made at the time of surgical decompression, so that all fractures were surgically compounded.

Presently there is no non-invasive method of measuring absolute bone blood flow in man. The measurement of bone uptake of fluorine-18 by positron emission tomography has

been reported as giving a measure of blood flow to vertebral bone, but only if combined with arterial blood sampling (Nahmias *et al.* 1986). Laser doppler flowmetry (LDF), the measurement of the doppler shift imparted to a laser beam by its reflection from moving blood cells (Swiontkowski *et al.* 1986), gives an index of cell flux as an electrical voltage, but no absolute figure for flow can be derived, and when applied to the measurement of tissue blood flow no differentiation is possible between the arterial or venous side of the circulation. Using this technique, Sanders *et al.* (1987) reported a comparative study of delayed unions of different long bones undergoing operative intervention. The *hypertrophic* type, character- ised by an 'elephant's foot' appearance on radiographs in which new bone is produced at the fracture margins but not across the fracture site, were found to have significantly higher red cell fluxes at the bone ends than the *atrophic* type, in which no attempt at healing is apparent (Figure 1.1.2). The uptake by bone of the scanning agent methylene diphosphonate has been found to correlate with bone blood flow during the first minute (Nutton *et al.* 1984, Nutton *et al.* 1985), and over longer periods if flow is in its normal range or decreased (Siegal *et al.* 1976, Sagar *et al.* 1979, Riggs *et al.* 1984), but neither early nor later scans have been found to correlate closely with the time to union (Gregg *et al.* 1983, Gregg *et al.* 1986, Oni 1987, Oni, Graebe and Gregg 1988).

An attempt to predict delayed union by visualisation of the bone vasculature has been made using osteo- medullography, the injection of contrast medium into the tibial marrow to give indirect information concerning the intramedullary circulation (Puranen and Kaski 1974). These workers, in a selected group of patients, found that the passage of medium into the veins of the proximal fragment after injection into the distal fragment was associated with subsequent consolidation of the fracture, but this may be related to the presence or otherwise of a barrier to union rather than to the level of bone blood flow.

Thus, while there is qualitative experimental evidence to support the importance of blood supply in long bone healing, there is little evidence available from studies in man.

It follows from the vascular hypothesis of delayed union that management of tibial shaft fractures should be tailored to maximise the vascular response, but at present there is little quantitative information as to the relative importance of the soft tissue and of the marrow circulation in this process, and there is no experimental evidence that increased vascular damage in higher energy fractures is associated with a significant reduction in the subsequent bone blood flow. The measurement of this flow is made difficult by the complicated vascular arrangement in bone, the functional details of which remain unsettled.

(a) transverse   (b) oblique   (c) spiral

(d) butterfly   (e) segmental
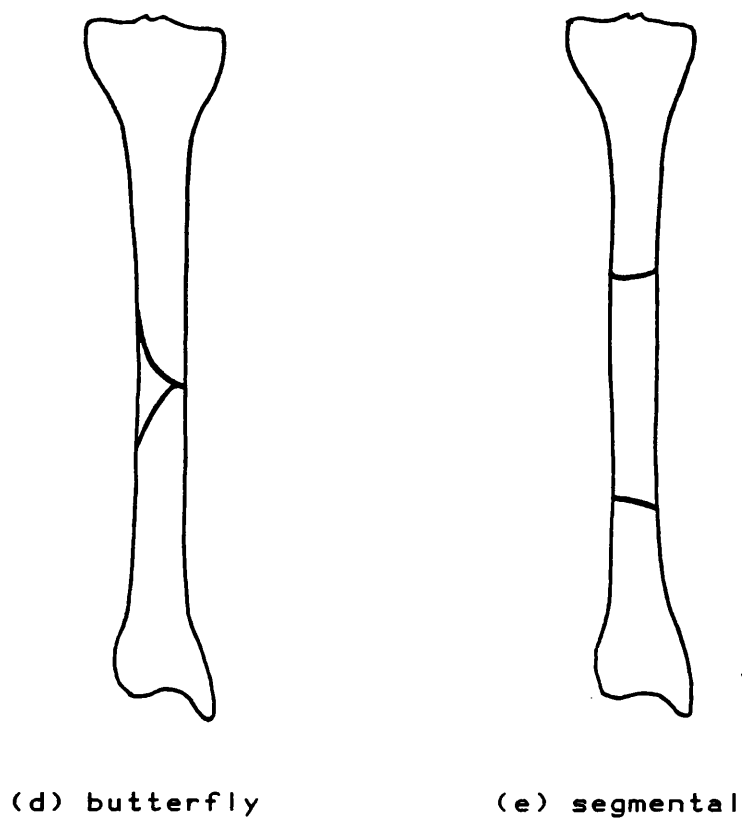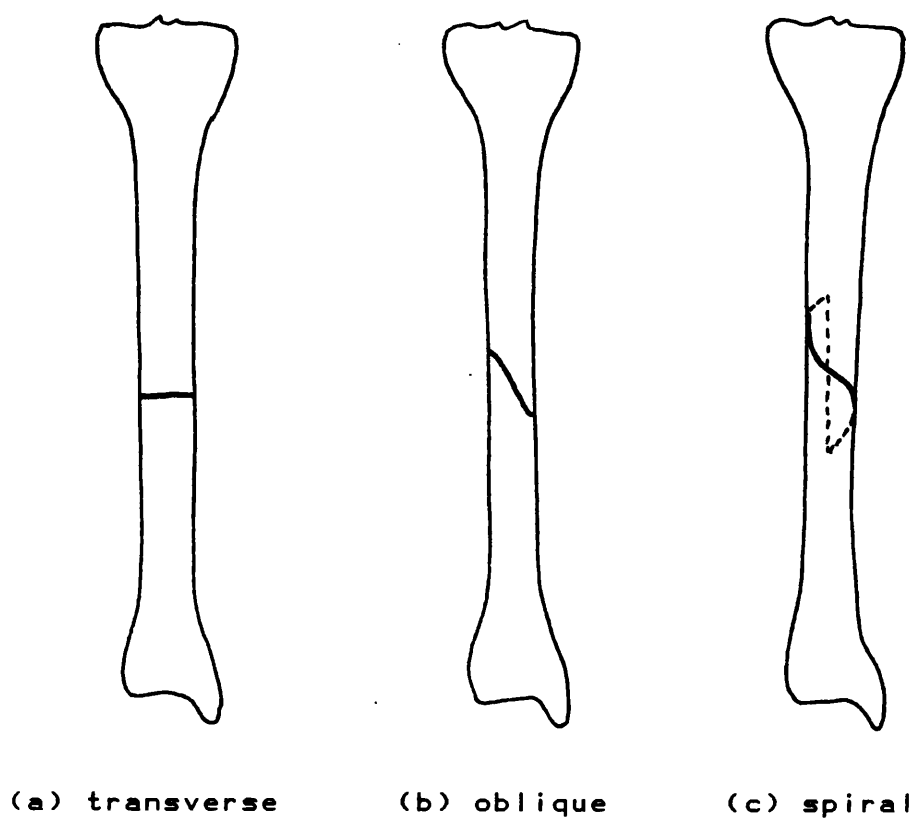
Figure 1.1.1   Patterns of fracture of the adult tibial
              diaphysis (after Johner and Wruhs 1983).

Figure 1.1.2
Radiographs of hyper-
trophic and atrophic
delayed union of the
tibial diaphysis.

(a) hypertrophic

(b) atrophic

1.2

THE INVESTIGATION OF THE BLOOD SUPPLY OF LONG BONES

The methods available for the investigation of the circulation of long bones fall into the two main groups of form and function, *ie* those demonstrating the arrangement of the blood vessels, and those giving an estimate of the blood flow under varying conditions. The quantitative methods are closely dependent on the anatomical studies, as the interpretation of the figures obtained will rely on the understanding of the vascular relationships of neighbouring tissues. The principal methods of bone blood flow estimation have been radio-isotope clearance, hydrogen or iodo-antipyrine washout, and the microsphere technique. The anatomy of the osseous circulation has been studied by vascular perfusion studies.

1.2.1 PERFUSION STUDIES OF THE BLOOD SUPPLY OF LONG BONES

The blood vessels to and within long bones may be demonstrated by intravascular injection with opaque dyes followed by clearance of the tissues for microscopy by the Spalteholz technique (1911). An alternative is the use of radiological contrast media as the injectate, followed by radiography of cut sections of bone. With either technique, which vessels are demonstrated will depend upon the injection pressure, the viscosity of the injectate, and the size of the injected particles. In particular, capillary and venous filling after arterial injection will require an injectate of low viscosity and small particle size (of the order of the size of red cells, 7-8μm). Retrograde venous injection may be added to show the venous side of the circulation.

Using these methods, there is general agreement as to the main routes of blood supply to an adult long bone, and as to the principal vascular structures within the bone. However, controversy remains as to the functional relationships of these elements.

Three major sources of blood supply are described (Brookes and Harrison 1957, Morgan 1959, Göthman 1960a, Nelson *et al.* 1960, Brookes 1971). The *nutrient* circulation arises from one or more nutrient arteries that pass obliquely into the shaft. Once in the medulla, ascending and descending branches pass towards the metaphyses, giving off radial branches towards the cortex. The vessels of the *periosteal* network communicate with those of the cortex over its entire surface, and are supplied by longitudinal vessels connecting circumferential anastomoses around the extremities of the bone. The network is further supplied by vessels arising from the neighbouring skeletal muscle and by those passing along fascial planes perpendicularly to the bone surface (Simpson 1985). The *epiphyseo-metaphyseal* supply comprises multiple branches of the periarticular anastomoses. In the adult, the circulations of the epiphyses, metaphyses and diaphysis freely communicate (Göthman 1960a).

The venous drainage of the bone comprises multiple vessels situated in greatest numbers in the metaphyses. In the diaphysis emissary veins arise from the peripheral marrow and pass directly through the cortex to the systemic venous system (Lopez-Curto *et al.* 1980, Oni *et al.* 1987). A central venous sinus runs the length of the diaphysis, emptied by terminal branches passing out of the metaphysis and by one or more 'nutrient' veins leaving the diaphysis (Brookes and Harrison 1957, Morgan 1959). The sinus can therefore be described as constituting the tributaries of the nutrient vein, analogous to the branches of the nutrient artery (Nelson *et al.* 1960). These veins contain no valves, and thus may be filled by retrograde venous infusion (Brookes 1987). The principal direction of venous

drainage of the diaphyseal cortex, whether centrifugal from the endosteal aspect to the periosteal veins, centripetal, or a combination of the two, is the subject of dispute.

Within the diaphyseal medulla lie radial arrangements of the branches of the nutrient vessels and of sinusoids aligned around the central venous sinus. The sinusoids comprise a fenestrated endothelial wall lacking a basement membrane (Brookes 1987), and are joined to the central sinus by larger collecting sinusoids (de Bruyn et al. 1970). Within the cortex, the Haversian and Volkmann canals each contain one or two small vessels, most of which have walls of single-cell thickness with no muscular elements. The arrangement is predominantly of oblique vessels passing between the periosteal and endosteal surfaces, with connecting transverse anastomoses (de Bruyn et al. 1970, Brookes 1987). Vessels may be seen passing between the cortical and periosteal circulations, and between the cortical and medullary circulations.

Perfusion studies do not indicate the direction of flow within the vessels demonstrated, and this has given rise to much of the controversy as to the functional relationships of the vessels described. Brookes and Harrison (1957) compared the results obtained with post mortem arterial and venous injection of barium sulphate suspension (Micropaque) in the tibia and femur of the rabbit. The medullary sinusoids filled only after venous infusion, and only the inner cortical vessels were shown by antegrade infusion. From these findings it was concluded that the sinusoids were entirely on the venous side of the capillary bed, and that the cortex received no arterial supply from the periosteum. However, both findings may have been at least partly influenced by the relatively high viscosity of the contrast medium (a 50% suspension) which would be expected to reduce penetration of the smallest vessels. Further studies using retrograde injections of Thorotrast resulted in filling of vessels throughout the thickness of the cortex, and this supported the view that the periosteal

circulation drained the cortical lattice rather than supplied it. On the basis of these studies, these workers postulated a predominantly centrifugal system of blood supply, with the cortex supplied from the medulla and draining into the medullary sinusoids and the periosteal vessels, but predominantly into the latter. This view was supported by a further study in which unilateral division of the tibial and femoral nutrient vessels in the rabbit resulted after two weeks in marked filling of cortical vessels from the periosteal surface, thus demonstrating that the lack of this filling on the control side was not due to technical problems (Brookes 1960a). Similar findings were reported in human tibiae studied after amputation for peripheral vascular disease (Brookes 1960b).

From micropaque and Indian ink perfusion preparations of the human tibia, together with histological studies, Nelson *et al.* (1960) concluded that the periosteum gave only a small capillary supply to the outer cortex, as no arterioles could be observed entering from the periosteal side. A few arterioles were, however, seen arising from the nutrient circulation. They further concluded that the predominant direction of venous drainage was centripetal, as venules were seen accompanying the radial branches of the nutrient artery as they entered the cortex, while fewer vessels were seen traversing the cortex to the periosteum.

De Bruyn *et al.* (1970) perfused guinea pigs, rabbits and rats with intracardiac Indian ink under pentobarbitone anaesthesia, and examined the circulations of the femur, tibia and humerus. The branches of the nutrient vessels were seen to reduce to capillary size before entering the cortex, with only occasional branches to medullary sinusoids lying peripherally. The cortical lattice communicated freely with both the sinusoidal system and with periosteal capillaries, and direct anastomoses between peripheral sinusoids and the periosteal network were seen. A further observation was that the sinusoids were well demonstrated only in sections where the cortical vessels

were also well filled. These findings lent support to the concept of the medullary sinusoid system as an essentially venous structure, but also indicated that the cortex received at least part of its arterial supply from the periosteum.

Rhinelander (1968) perfused the radii and ulnae of dogs with 30% Micropaque, but found that cortical filling was sparse unless the circulation was stimulated by a fracture of the other limb. Under these pathological circumstances, the outer one-quarter to one-third of the cortex appeared to be filled from the periosteal network, and there was free anastomosis between the medullary and periosteal cortical supplies. However, these conclusions were based on the premise that no venous filling was present, and this is unlikely in the presence of the claimed capillary filling.

Trias and Frey (1979) carried out *post mortem* perfusion of the tibial nutrient artery in dogs using Indian ink. They described nutrient vessels entering the cortex transversely and branching in its middle third. This corresponds with the finding of an increased density of cortical vessels seen at this site by Brookes and Harrison (1957) using retrograde Thorotrast infusion. Most of the cortical structures that were thought to be venous were seen to pass between the cortex and medulla, suggesting a predominantly centripetal venous drainage.

Lopez-Curto *et al.* (1980) used particles of silicone rubber (Microfil) sized 0.1 to 5.0µm to infuse the tibias of dogs under anaesthesia. The infusion was pulsed at 70 cycles a minute, and at a pressure of 100 to 150mmHg, in order to mimic physiological conditions. The venous structures passing between the cortex and medulla were described as vessels passing through the cortex between peripheral sinusoids and the periosteal network, and thus it was concluded that the bulk of the venous drainage of the cortex and peripheral sinusoidal system was centrifugal. These workers also found direct connections

between nutrient vessels and sinusoids, and their findings are essentially similar to those of de Bruyn *et al*. (1970).

Thus, while there is much agreement over the structures that may be demonstrated by perfusion studies, there is dispute regarding the principal directions of flow between the medulla, cortex and periosteum. One approach to this problem is to observe blood flow *in vivo*, and this was performed in an elegant and careful experiment by Brånemark (1959). The vessels at the interface of the cortex and medulla of the rabbit fibula were observed microscopically under controlled conditions of temperature by grinding down the outer cortical layers. A parallel system of supply was described, with marrow arterioles branching into capillaries that supply medullary sinusoids or which enter the cortex. The sinusoids were drained by venules into the central sinus, while the cortical capillaries drained into peripheral sinusoids. According to this description, venous flow is centripetal, although the cortical findings apply only to the inner 10 to 30μm.

Another approach to the problem is to study the routes of supply separately by exclusion experiments. These give information as to the domains of the principal arterial sources, although these experimental domains are necessarily facultative rather than physiological.

Trueta and Cavadias (1964), following a modification of the method of Johnson (1927), studied the radius of the rabbit. The periosteal, nutrient and epiphyseo-metaphyseal supplies were excluded from the bone in pairs by the use of polythene sheets or plugs, and the bone examined radiologically, angiographically and histologically. In adult animals, the periosteal vessels, when left as the sole source of supply to the diaphysis, were unable to prevent cell necrosis in the inner half to two-thirds of the cortical thickness, and across virtually the entire marrow. The nutrient artery, however, supported a normal marrow, but not the outer one-quarter to one-third of the cortical cells. The epiphyseo-metaphyseal supply was able,

by virtue of its anastomoses with the intact intra-osseous parts of the nutrient vessels, to support the marrow and the inner cortex, although to a lesser extent than by the intact nutrient artery. Thus, division of the nutrient artery alone, leaving the other two supplies intact, was found to have no effect whatsoever upon the bone. The findings of these studies closely matched those of Johnson (1927) in the dog tibia.

Brookes (1987) differentiates the vessels of the cortical lattice into two types on the basis of light and electron microscopy. The bulk of the vessels are described as sinusoids, having the same structure as the medullary sinusoids, while vessels that are histologically terminal arterioles and are derived from the nutrient circulation join the lattice in the inner two-thirds of the cortex. Similar vessels are not seen from the periosteal side, although capillaries penetrate the outer cortex and appear to arise from periosteal arterioles (Nelson et al. 1960). These findings, together with the difficulty in filling cortical vessels from the periosteal circulation, are taken to indicate a purely nutrient supply to the cortex with a purely centrifugal venous flow. The inability of the nutrient vessels to support the outer cortex in exclusion experiments might thus be the result of venous infarction following the lifting of the periosteum, a possibility envisaged by Trueta and Cavadias (1964). However, the studies of Brånemark indicate that the medullary sinusoids drain blood from the inner cortex as well as receiving an arterial input, and, contrary to Brookes' assertion, a lattice is capable of supporting flows in differing directions within its different limbs, as Brånemark observed in the marrow. It thus remains possible that the venous drainage of the cortex is in both directions, with the predominant direction of flow determined by prevailing physiological and pathological conditions.

Anatomical studies using vascular perfusion allow two important conclusions. Firstly, the diaphyseal cortex does

not appear to be supplied by a portal circulation from the
marrow, although the evidence regarding the periosteal
capillaries is less clear. Thus it may be assumed that the
concentrations of tracers in precapillary arterial blood
are the same in the cortex as in the marrow. Secondly,
anastomoses are already present between the nutrient and
epiphyseo-metaphyseal arterial supplies, and between the
medullary and periosteal venous systems, and these may
assume particular importance after disruption by a
fracture.

In view of the multiple arterial and venous vessels
comprising the blood supply of a long bone, it is not
practicable to measure blood flow quantitatively by direct
methods such as by flowmeters or venous effluent
collection. Studies have relied therefore on the use of
tracers, using the principles of indicator clearance,
washout, or fractionation.

1.2.2 RADIO-ISOTOPE CLEARANCE STUDY OF BLOOD FLOW IN LONG BONES

This technique measures the uptake of an indicator into the
bone from the blood after a bolus injection. By the Fick
principle, tissue flow equals the indicator content of the
tissue divided by the integral of the arteriovenous
difference in indicator blood concentration across the
tissue. Dividing each side of the equation by the volume of
the tissue gives :

$$Q_{tissue} = I_{tissue} / \int (I_a - I_v) \, dt \qquad (1.1)$$

where Q_{tissue} = tissue blood flow per unit volume

I_{tissue} = tissue concentration of indicator

I_a = arterial concentration of indicator

I_v = venous concentration of indicator

The *clearance* of the indicator is a virtual figure that
represents the amount of arterial blood that is completely
relieved of its tracer in unit time :

$$C_{tissue} = I_{tissue} \ / \int I_a \ dt \qquad\qquad (1.2)$$

where C_{tissue} = tissue clearance of tracer per unit volume.

This obviates the requirement of representative venous
sampling. If the extraction of the tracer is complete, so
that its venous concentration is zero, then the clearance
by a tissue is equal to its blood flow. In practice,
extraction is incomplete, and the *extraction ratio* is less
than 100% :

$$E_{tissue} = \int(I_a \ - \ I_v) \ dt \ / \int I_a \ dt \qquad\qquad (1.3)$$

where E_{tissue} = tissue extraction ratio. Substituting for
$\int(I_a \ - \ I_v) \ dt$ from equation 1.1 gives :

$$E_{tissue} = I_{tissue} \ / \ (Q_{tissue} . \int I_a \ dt) \qquad\qquad (1.4)$$

$$\text{or} \qquad E_{tissue} = C_{tissue} \ / \ Q_{tissue} \qquad\qquad (1.5)$$

The extraction ratio for the tissue must be known for a
blood clearance to be corrected to a blood flow. Many
studies, however, assume a ratio of 100%.

The tracers may be diffusible ions such as potassium-42
and rubidium-86 (Kane and Grim 1966, 1969), or ions that
are taken up specifically by mineralised bone, the so-
called *bone-seeking* isotopes. These have included
calcium-47 (Weinman *et al.* 1963), calcium-45 (Shim *et al.*
1967, Bosch 1969, Schoutens *et al.* 1979), fluorine-18
(Wootton 1974, Lemon *et al.* 1980, Wootton and Doré 1986,
Nahmias *et al.* 1986), and strontium-85 (Copp and Shim 1965,

Shim *et al.* 1967, Shim *et al.* 1968, Sim and Kelly 1970, Cofield *et al.* 1975, Lemon *et al.* 1980, Tothill and Hooper 1984).

The technique has the advantage of avoiding surgical exposure of the bone, although this is as a result of the technical impracticality of collecting representative osseous venous samples. It also allows the study of more than one bone simultaneously. However, it suffers from several major disadvantages.

1. Only one measurement is possible per animal. The isotopes are subject to back-diffusion into the blood both during and after the experiment (Copp and Shim 1965, Cofield *et al.* 1975, Lemon *et al.* 1980), and the preparation is therefore unstable.

2. Marrow flow cannot be estimated by bone-seeking isotopes. Bone-seeking isotopes have a negligible clearance by the non-calcified marrow (Bosch 1969, Wootton 1974).

3. Tracer diffusion continues post mortem. The indicator is not fixed, and is able to move into the bone from the surrounding tissues *post mortem* until the bone is excised and cleaned. This is a particular problem with bone-seeking isotopes. Tothill and MacPherson (1978) found the bone contents of Sr^{85}, Ca^{47} and F^{18} to increase progressively during the first hour after death in rats and rabbits. For the period commencing three minutes after death in the rabbit, Wootton and Doré (1986) found on the basis of a linear analysis that the apparent extraction ratio of F^{18} increased at the rate of approximately 0.01 per minute while the bone remained *in situ*. This is likely to be an underestimation, as about three-quarters of the measurements used to calculate this figure were obtained beyond ten minutes after death, and the rate of *post mortem* uptake will probably be greatest in the first few minutes while local extra-osseous tissue levels of tracer are high.

The uptake of tracer by bone before death may be measured by the use of external counters, but these measurements will be affected by counts in the surrounding tissue, which may be substantial even with bone-seeking isotopes. After intravenous injection in dogs, 40% of Sr^{85} was found in skeletal muscle at ten minutes (Sim and Kelly 1970).

4. Counts of bone radioactivity include indicator remaining in blood within the bone. This effect results in an overestimation of bone uptake, particularly if the extraction ratio is low. The blood volume for tibial and femoral cortex is of the order of 0.33 ml/100g in the dog, while in cancellous bone it approximates 3.0 ml/100g (Tøndevold and Eliasen 1982b), representing approximately 0.6 to 6% of bone volume. An attempt has been made by Wootton and Doré (1986) to estimate the amount of F^{18} remaining in the blood vessels of the rabbit tibia and femur by the use of erythrocytes labelled with chromium-51, in an experiment where recirculation was almost completely prevented. Their calculation did not include any figures for bone F^{18} activity, and no derivation of their equation is given. According to their figures, more than half the fluorine in these bones lay intravascularly, but this appears high in view of their claim of an extraction ratio approximating unity.

5. The transit times for the bone circulation probably exceed the minimum systemic recirculation time. To calculate clearance, the amount of tracer in the arterial blood presented to the tissue of interest must be known (equation 1.2). This integral is calculated from withdrawal samples taken from a major artery away from the limb. However, as a result of differences in local transit times, these samples may not match the arterial blood in bone.

Tothill and MacPherson (1980) found the minimum systemic recirculation time in the rabbit to approximate ten seconds, while labelled erythrocytes injected into the abdominal aorta with recirculation effectively prevented

were still found in the rabbit femur and tibia 45 seconds
after injection (Wootton and Doré 1986). The minimum
recirculation time in the dog is under one minute (Kane and
Grim 1969), but albumin injected into the tibial nutrient
artery of the dog continues to be washed from the bone for
some two to four minutes (Cofield et al. 1975, Lemon et al.
1980). This time will have been prolonged to an unknown
extent by leakage of the albumin into the extravascular
space. Tothill and Hooper (1984) attempted to show in rats
and rabbits that the proportion of labelled 'intravascular'
albumin in bone did not fall to negligible amounts before
rising again from recirculation, but the small proportions
of the cardiac output distributed to single bones, and the
very short minimum recirculation times in these animals,
makes accurate measurements against time very difficult.

Kane and Grim (1969) claim that at one minute after
intravenous injection in the dog the amounts of K^{42} and
Rb^{86} delivered to the tissues as a result of recirculation
equal those lost into the venous outflow. In other words
the isotopes are in equilibrium between tissues and blood.
From equation 1.1 :

$$Q_{tissue} = I_{tissue} / \int (I_a + I_{ar} - I_v)\, dt \qquad (1.6)$$

where I_{ar} is the arterial concentration of indicator
arising from recirculation. At equilibrium, I_{ar} equals I_v
and comparing with equation 1.2 it can be seen that flow
now equals clearance (Kane 1968). To obtain $\int I_a\, dt$ the
downslope of the arterial dilution curve is extrapolated
beyond the point where it is interrupted by recirculating
tracer, and the area under the resulting curve measured.
Using this method, these workers found that the isotope
clearances for the isolated canine hind limb closely
matched the total venous outflow. However, the technique
assumes both that the extraction ratios of different
tissues are the same, and that all tissues are in

equilibrium with the isotope. This may cause errors when flows to individual tissues are measured, although good agreement was found between the distributions to bone of Rb^{86} and 16µm or 25µm glass microspheres (discussed below in Section 1.2.4).

Bone-seeking isotopes do not appear to have been investigated along similar lines. Rather, the problem posed by recirculation has led to efforts to measure extraction ratios of these isotopes during their 'first pass' through the bone circulation.

One method is to inject the tracer into the tibial nutrient artery and divert all the osseous venous outflow to a cannula in the femoral vein by the use of a tourniquet. Thus recirculation is prevented. Correction for the dilution of the tracer by venous blood from other parts of the hindlimb can be made by the addition of an intravascular marker to the injectate (Copp and Shim 1965, Bosch 1969, Cofield *et al.* 1975, Lemon *et al.* 1980). However, injection into the nutrient artery, with or without occluding this vessel, results in the measured ratios applying to only part of a disturbed circulation. Further, the 'intravascular' marker in these studies has been albumin, which has been shown to have a significant extravascular volume of distribution in bone within ten minutes of its administration (Tøndevold and Eliasen 1982b). Apart from these problems, the ratios are found to vary with time after injection, possibly as a result of exchange with more than one osseous compartment (Bosch 1969).

An alternative is to stop the circulation before recirculation can occur, but as recirculation time will vary with changes in cardiac output and tissue flow, and extraction ratio varies with time after injection, these measurements are of questionable value. Tothill and MacPherson (1980) compared the proportions of F^{18}, Sr^{85} and labelled microspheres distributed to the tibia and femur of rabbits and rats within the minimum systemic recirculation

times, but this calculation suffers from the further disadvantage of not accounting for the avidity of bone mineral for the ionic tracers which does not affect microsphere distribution. Wootton and Doré (1986) undertook the same comparison for F^{18} in the rabbit while surgically preventing recirculation. These workers found extraction ratios of unity or greater using corrections for *post mortem* migration of tracer, although, as discussed above, this was probably underestimated.

As a result of the apparently long transit times in bone, the estimation of extraction ratios in the absence of recirculation will not allow accurate correction of measured clearances to bone flows on the basis of arterial sampling unless the experiment in question also excludes recirculation. In general, in the application of the clearance technique to investigate osseous vascular pathology, the disturbances required for this exclusion will be better avoided.

6. Extraction ratios may vary with flow rate. From the above discussion, it can be seen that unless the tracer is in equilibrium between blood and bone, the apparent extraction ratio will vary with time even with a constant flow rate. Changes in flow are likely to affect the time course of changes in the ratio. In addition, at high flow rates, uptake of tracer by bone may become limited by factors other than flow, so that the flow is no longer reflected by the tracer clearance. Such factors may include the transfer of bone-seeking tracers into bone mineral from the extravascular bone fluid (Charkes *et al.* 1979) or the opening up at higher flows of arteriovenous shunts (Friedman 1968).

Estimates of the extraction ratios of Ca^{45}, F^{18} and Sr^{85} by the method of nutrient artery injection and collection of femoral venous blood in the dog have shown small drops in the ratios with increasing flow, over small ranges of the latter (Bosch 1969, Cofield *et al.* 1975, Lemon *et al.* 1980). A more marked effect was seen over a

wider range of flows with comparison of Sr[85] clearance with flows estimated by the microsphere technique (Tothill *et al.* 1985). Schoutens *et al.* (1979) found the clearance of Ca[45] to approximate 50% of the flow obtained with 15μm microspheres under control conditions in the whole tibia and femur of the rat. Bone blood flow was varied over a wide range by raising and lowering the ambient temperature of the hindlimbs, and the extraction ratio of the tracer in the tibia dropped from 0.77 to 0.27 with increase in flow from below six to above twelve ml/min.100g. Thus increases in flow above control levels was not matched by increases in tracer clearance.

In summary, the measurement of bone blood flow by clearance methods is subject to inaccuracies resulting from the avidity of bone for many of the tracers used, and from the apparent variations in extraction ratios. These ratios are difficult to quantify, particularly if the isotope is not in equilibrium between blood and bone. The problems posed by blood-bone tracer exchange also apply to another method of flow measurement, indicator washout.

1.2.3 INDICATOR WASHOUT STUDY OF BLOOD FLOW IN LONG BONES

In principle, the measurement of blood flow by the washout of a tracer is the reverse of a clearance measurement. The disappearance of the tracer from a tissue is assumed to be the result purely of uptake into the blood perfusing that tissue, and has the general form of exponential decay :

$$I_t \ = \ I_0 e^{-kt} \qquad\qquad\qquad (1.7)$$

where I_t = tissue concentration of tracer at time t

I_0 = tissue concentration of tracer at zero time

e = the base of natural logarithms

and k is a constant

The constant k may be obtained by a semilogarithmic plot of I_t against time :

$$\log_e I_t = \log_e I_0 - kt \qquad (1.8)$$

This has the form of a linear equation with k as the slope.

Two simplifying assumptions are made. Firstly, it is assumed that the arterial concentration of the indicator is zero. Secondly, it is assumed that the tissue tracer is in equilibrium with end-capillary blood, and thus with venous blood. Under these conditions, the 'extraction ratio' is constant, and is expressed as a *partition coefficient* (λ) of the tracer between tissue and blood.

$$\lambda = I_t / I_v \qquad (1.9)$$

An expression for blood flow may be obtained by the reverse of the Fick principle (equation 1.1) :

$$Q_{tissue} = I_0 - I_t / \int (I_v - I_a) \, dt \qquad (1.10)$$

By substituting I_t / λ for I_v (equation 1.9) and equation 1.7 for I_t this may be reduced to :

$$Q_{tissue} = k\lambda \qquad (1.11)$$

The tracers that have been used for the study of bone blood flow have included radio-iodide (Brown-Grant and Cumming 1962), radiolabelled iodo-antipyrine (Kelly *et al.* 1971, Semb 1971, Kelly 1973, McElfresh and Kelly 1974), xenon-133 (Semb 1971, Lahtinen *et al.* 1979), and hydrogen (Whiteside, Lesker and Simmons 1977, Whiteside, Simmons and Lesker

1977, Whiteside *et al.* 1978, Weiland *et al.* 1982, Kita *et
al.* 1987). Most tracers are administered by local
injection, and the tissue levels measured by external
counting of the isotope label. This method gives the
fractional disappearance of the tracer rather than the fall
in tissue concentration, but this is simply equivalent to
dividing equation 1.7 on both sides by I_0. Hydrogen may be
administered with the inspired gases, and subsequently
measured locally by the use of a platinum tissue electrode
(Aukland *et al.* 1964).

The value for λ for each tissue may be obtained by
measuring uptake into a sample of tissue placed into a
solution of the tracer *in vitro* (Aukland *et al.* 1964,
Whiteside, Lesker and Simmons 1977). The finding that small
sections of bone do not take up any hydrogen from
surrounding muscle *in vivo* (Whiteside, Lesker and Simmons
1977) suggests, however, that in this tissue the 'uptake'
during the measurement of λ is by exchange of water between
the bone fluid and the hydrogen solution rather than by
simple hydrogen diffusion. Possibly this is the main
mechanism of exchange of tracer between tissue and blood.

Kelly's group has given estimates of the coefficient
for iodo-antipyrine in bone on the basis of measurements
made during nutrient artery infusion *in vivo* (Kelly *et al.*
1971, Kelly 1973), but the problem of obtaining a
representative sample of osseous venous blood to give I_v
(equation 1.9) means that these figures must be viewed with
caution.

Measurement of tracer washout overcomes some of the
problems of the clearance method. Firstly, once the tracer
has been washed out of the tissue, a repeat measurement may
be made. Secondly, flow to the marrow may be estimated if
the appropriate partition coefficient is known. Thirdly, as
the measurements are made purely *in vivo*, movements of
tracer *post mortem* are irrelevant. Indeed, there is no need
to sacrifice the animal, and thus further measurements may
be made at some remote time. However, the problems posed by

tracer situated in blood within the tissue, recirculation, and the time course of blood-tissue exchange remain.

1. Counts of bone radioactivity include tracer within blood and other tissues. Whichever method is used, the tracer that has passed into the capillary blood will be measured in addition to that remaining in the tissue. Electrodes inserted into bone will be surrounded by blood mixed with tissue fluid, and external counters will pick up emissions from both blood and non-osseous tissues.

Attempts have been made to avoid the presence of tracer in non-osseous tissue by the use of local injection, either directly into the bone (Brown-Grant and Cumming 1962, Semb 1971, Lahtinen *et al.* 1979) or into the nutrient artery (Kelly *et al.* 1971, Kelly 1973, Lavender *et al.* 1979). Both methods involve local trauma, and the latter distributes tracer to only part of the bone circulation while at the same time causing interference with that circulation. Injection of iodo-antipyrine into the anterior tibial artery of dogs near the nutrient artery, rather than directly into it, resulted in only 20% reaching the tibia, with the remainder found in the nearby muscle (McElfresh and Kelly 1974).

A further potential problem with external counting is that the washout curves for cortex, muscle and marrow are superimposed. In the dog tibia, however, the curves for iodo-antipyrine in cortex, surrounding muscle and marrow have been found to be the same (Kelly *et al.* 1971, McElfresh and Kelly 1974).

2. Recirculation may result in an arterial concentration of tracer above zero. Any recirculation will slow the net washout of the tracer. Aukland *et al.* (1964) used intra-arterial electrodes during hydrogen washout measurements in the dog kidney, and found that femoral arterial levels of inspired hydrogen took approximately 40 seconds to drop to negligible amounts. Measured kidney cortical blood flow matched its venous outflow only after this time period.

Whiteside, Lesker and Simmons (1977) found the equivalent time in the rabbit to be 20 seconds. The apparently long transit times in bone will exaggerate this effect, which may be sufficient to obscure any early fast component of the washout (Aukland *et al.* 1964).

Kelly *et al.* (1971) found negligible levels of external emission over the contralateral tibia in dogs after nutrient artery injection of iodo-antipyrine, suggesting that local injection of small amounts of tracer resulted in insignificant recirculation. Hughes *et al.* (1978), after a similar injection of radiolabelled methylene diphosphonate, found the radioactivity of the contralateral tibial shaft to be four to five per cent of that of the injected tibia after five minutes, but any recirculation of this bone-seeking tracer will be concentrated in bone, unlike antipyrine.

3. Tissue tracer may not be in equilibrium with end-capillary blood. As with the clearance technique, problems are posed by the time-course of equilibration and the possibility of diffusion limitation of exchange at high flow rates. This is unlikely with hydrogen, which is lipophilic and of a very low molecular weight, but data is lacking for the other tracers.

4. Measurement of tracer by tissue electrodes may be subject to diffusion limitation within the surrounding tissue. The tissue immediately surrounding an electrode may be subject to circulatory disturbance that will slow the washout of the tracer. At low flow rates, diffusion of the tracer across this tissue from well-perfused areas will be sufficient for the *rate* of washout to be measured accurately, albeit after an initial delay. However, at high flow rates this diffusion may be limiting. The high diffusibility of hydrogen results in this effect being apparent only with flow rates above 150 ml/min.100g (Aukland *et al.* 1964). In bone, Whiteside, Lesker and Simmons (1977) found that the vessels adjacent to the drill holes made for the electrode remained patent on

histological sections, but this gives no indication of the
rate of flow in these vessels with respect to the remainder
of the bone.

5. Multiexponential washout. Washout curves with more than
one component have been found in the kidney, skeletal
muscle, femoral cancellous bone, and tibial diaphyseal
marrow (Aukland *et al.* 1964, Semb 1971, Whiteside, Lesker
and Simmons 1977, Lahtinen *et al.* 1979). This effect may
arise from heterogeneous tissue blood flow, or from
diffusion limitation of movement of tracer from one or more
tissue compartments. Again, the latter explanation appears
unlikely in the case of hydrogen, particularly as
circulatory arrest results in abrupt cessation of washout
(Whiteside, Lesker and Simmons 1977) which would be
expected to continue for a short period if it is not
entirely flow limited.

Heterogeneity of blood flow will have important effects
on the distribution of tracer after its administration.
Aukland *et al.* (1964) found that the shape of hydrogen
desaturation curves of kidney cortex was dependent on the
duration of hydrogen respiration used for the initial
saturation. In the case of tracers administered by local
intravascular injection, those areas of tissue with very
low flows may not have time to take up significant amounts
of tracer before washout exceeds uptake. In this way, the
average tissue flow may be overestimated. The direct
injection of tracer may be less subject to this effect.

In summary, the use of tracer washout to measure bone
blood flow thus has some advantages over the clearance
technique, while retaining some of its problems. The
necessity to measure tissue levels of tracer *in vivo* adds
specific disadvantages, those of the local trauma of tissue
electrodes or of the use of external monitoring. Much of
the difficulty of the interpretation of tracer movements
between tissue and blood relate to the time course of such
movements, particularly in relation to recirculation. These
problems may be overcome by the use of a flow indicator

that does not leave the circulation while at the same time undergoing physical integration of its distribution. These properties are supplied by radioactive microspheres.

1.2.4 THE STUDY OF BLOOD FLOW IN LONG BONES WITH RADIOACTIVE MICROSPHERES

According to the indicator fractionation principle, particles injected into the left side of the heart will be distributed throughout the body in proportion to the distribution of the cardiac output. If on first pass they impact in the precapillary vessels and capillary bed, then physical integration of the 'dilution' curve results (Wagner *et al.* 1969, Heymann *et al.* 1977). Tissue blood flow is therefore simply calculated as :

$$Q_{tissue} = Q \ . \ (N_{tissue} \ / \ N_{total}) \hspace{2cm} (1.12)$$

where Q = cardiac output

N_{tissue} = number of particles in the tissue

N_{total} = total number of particles injected

If the particles, or microspheres, are labelled with a radio-isotope, and if the frequency distribution of radioactivities of the microspheres is the same in the tissue as in the injectate, this calculation may be expressed as :

$$Q_{tissue} = Q \ . \ (Count_{tissue} \ / \ Count_{total}) \hspace{1.5cm} (1.13)$$

where *Count* represents the radioactivity count. Cardiac output may be estimated by an independent method, or by the use of a surrogate 'organ' of known flow. If a reference sample is withdrawn from a major artery at a known rate during the distribution of the microspheres, then :

$$Q_{ref} = Q \cdot (Count_{ref} / Count_{total})$$ (1.14)

where the subscript *ref* refers to the reference sample. Solving for Q gives :

$$Q = Q_{ref} \cdot (Count_{total} / Count_{ref})$$ (1.15)

Substituting equation 1.15 for Q in equation 1.13 :

$$Q_{tissue} = Q_{ref} \cdot (Count_{tissue} / Count_{ref})$$ (1.16)

Thus, the number of microspheres injected does not need to be known.

Using this method, no surgical interference with the bone or its blood supply is required, and the problems inherent in methods relying on blood-tissue tracer exchange are avoided. It relies on three assumptions.

1. It is assumed that the microspheres are sufficiently evenly mixed in the arterial blood to be distributed in proportion to regional flows. This requires both adequate mixing at the injection site and absence of significant arterial streaming. If cardiac mixing is complete or nearly so, then flows to paired organs should be equal, and this has been found in the kidneys (Neutze *et al.* 1968, Mendell and Hollenberg 1971, Sasaki and Wagner 1971, Warren and Ledingham 1974, Malik *et al.* 1976), cerebral hemispheres (Sasaki and Wagner 1971), and hindlimb bones (Syftestad and Boelkins 1980, Jones *et al.* 1982). Morris and Kelly (1980) found that evenness of mixing to the limbs was less complete in supine dogs than in those conscious and standing, although no figures were given.

A further test of central mixing is to compare samples of blood taken from different major arteries, which should

contain similar numbers of microspheres regardless of their site. This has been confirmed in the dog (Morris and Kelly 1980, Moore *et al*. 1981) and in the rabbit (Neutze *et al*. 1968, Syftestad and Boelkins 1980). Kaihara *et al*. (1968) found no differences in the distribution of microspheres labelled with two different isotopes and injected simultaneously.

Streaming of microspheres within the aortic arch has been found with the larger sizes of microsphere (50μm) in the rabbit, where these spheres were preferentially distributed to the head (Warren and Ledingham 1974). They also tend to be distributed towards the centre of the lumens of smaller arteries (Phibbs and Dong 1970), reducing the likelihood of their entering small side-branches. Only microspheres of 10μm or less were found to be evenly distributed, and thus the use of larger spheres is likely to introduce a small error at this level of the circulation.

2. It is assumed that there is no recirculation of microspheres. Recirculating microspheres will be re-distributed to the tissues in proportion to regional blood flow, where on subsequent passes they may be trapped, but inaccuracy in their final distribution will result if the degree of non-entrapment on each pass varies between different regions.

Non-entrapment arises from passage through arterio-venous shunts and through the capillary bed, if the microspheres are sufficiently small. In the case of shunts, flow through these channels is not, by definition, measured by clearance or washout methods either, and the measured flow is that of the 'nutritive' capillary circulation. Thus, shunting presents a problem with the microsphere technique only if the shunted microspheres are not trapped by the pulmonary circulation and are allowed to recirculate. Zanick and Delaney (1973) injected microspheres sized 18 to 34μm into the femoral arteries of dogs and found 4% of the dose in the lungs but no spheres

in the kidneys, confirming the lack of pulmonary shunting of this size range. Kaihara *et al.* (1968) found 99.7% of an intravenous dose of 15 ± 5 μm (SD) microspheres trapped in the lungs in the dog, while Warren and Ledingham (1974) did not detect any pulmonary shunting of similar spheres in the rabbit. By sampling arterial blood after right atrial injection in dogs, Fan *et al.* (1979) obtained pulmonary shunting rates of less than 1% for 15 ± 1 μm spheres and 3.5% for spheres of 9 ± 0.6 μm. Thus it appears that recirculation is insignificant for microspheres of greater diameter than approximately 9μm, a result which might be expected on the basis of the size of erythrocytes.

Microspheres of less than this size passing through peripheral capillary beds are unlikely to be trapped in the lungs, and this will lead to inaccuracies in the calculation of blood flow. The extent of non-entrapment in any tissue is best assessed by examination of the size distribution of the microspheres in a representative venous sample. In the kidney, no spheres of 10μm or greater pass into the renal vein (Katz *et al.* 1971, Archie *et al.* 1973) and the same is true in isolated skeletal muscle (Gaehtgens *et al.* 1976). In human placental cotyledons *in vitro*, less than 5% of microspheres larger than 10μm pass (Penfold *et al.* 1981). Thus, the assumption of complete trapping of the microspheres in the capillary circulation has been found to be reliable in soft tissue studies if the microspheres are all greater than 10μm in size.

In bone, representative venous sampling is not possible, and non-entrapment has been assessed both by nutrient artery injection and by comparison of simultaneous calculated flows. Measurements after tibial nutrient artery injection have been carried out only with 15μm microspheres, and suffer the usual disadvantages of this approach. Morris and Kelly (1.980) found over 14% of the injectate appearing in the femoral vein in a single experiment, while Tothill, Hooper, Hughes and McCarthy (1987) found a rate of 0.86%. The higher figure of the

former workers might be explained by the finding of Tothill
et al. that non-entrapment was increased to 20% or more by
previous ischaemia. Gross *et al.* (1979) obtained counts in
the tibial nutrient vein amounting to 1% of those in
arterial samples after intracardiac injection.

Comparison of flows to bone obtained with
simultaneously injected microspheres of different sizes
have been used to assess the relative degree of non-
entrapment of the smaller spheres. To date, these
comparisons have been confined to 15μm and 9μm microspheres
(Niv and Hungerford 1979, Gross *et al.* 1979, Jones *et al.*
1982), and have shown calculated blood flows 30 to 50%
lower with the smaller spheres in samples from the femoral
head and in mixed bone samples. These results suggest a
considerable degree of non-entrapment of 9μm microspheres,
as would be expected from the soft tissue studies.

While small microspheres are more likely to behave in
the manner of erythrocytes in the arterial circulation,
non-entrapment studies suggest that a minimum size of 10μm
is required to avoid inaccuracies arising from
recirculation.

*3. It is assumed that the frequency distribution of the
radioactivities of the microspheres in the tissue is the
same as that in the injectate.* Microspheres may be
labelled by an isotope coating or by incorporation of
isotope into the substance of each sphere. In the first
case, the radioactivity of each sphere will approximate a
function of the square of its radius, while in the second a
function of the cube. It can be seen that small variations
in size of the microspheres will be associated with large
variations in their activity. For example, a batch of
microspheres sized 15 ± 1 μm with incorporated isotope will
have a size range of the order of 12 to 18μm. Very few
spheres will be of the extreme size, but limiting
consideration to those of 13 to 17μm still implies that the
largest spheres will carry more than twice the amount of
isotope as the smallest. This will not affect the

calculation of blood flow (equation 1.16) unless the frequency distribution of the microsphere size in the tissue is different from that in the injectate, and therefore also from that in the reference sample.

Such a difference will arise from non-entrapment of the smallest microspheres, or from hindrance of the entry of larger spheres into the tissue sample. This *steric restriction* (Mørkrid *et al.* 1976) will arise at the microvascular level, and is unlikely to affect the measurement of flow to whole organs. An extreme example of the effect is the case of a postcapillary circulation, such as that of the renal medulla (Katz *et al.* 1971). Here, any microspheres small enough to reach the medulla pass through into the venous blood, and the tissue count rates are negligible. Within the cortex, the site of microsphere trapping is dependent on the microsphere size, so that while almost all 15μm spheres are trapped in the glomeruli, virtually all 35μm spheres lodge elsewhere in the larger vessels. The corollary of this is that the calculated local tissue flow rates at different depths within the cortex will vary according to the size of microsphere used – the larger spheres may lie outside the tissue whose flow they represent.

In order for the third assumption to be valid for the measurement of blood flow within an organ, therefore, the microspheres must be sized as tightly as possible, close to but greater than 10μm so as to avoid non-entrapment while minimising steric restriction.

While the three basic assumptions of the microsphere technique may be satisfied, it must also be shown that the embolisation caused by microsphere injection does not adversely affect the circulation, by effects either on the central nervous system or on the local microvasculature. Central effects have been found to be more common in conscious animals, and with both administration of large numbers of microspheres and increasing microsphere size (Warren and Ledingham 1974). Such effects include transient

hypotension and bradycardia. In the anaesthetised rabbit, between 2 and 100 × 10⁶ spheres of 15μm have been injected without apparent central effects (Hierton 1983, Aalto and Slätis 1984, Davis *et al.* 1990), while in the dog up to 157 × 10⁶ have been given (Morris and Kelly 1980).

Local effects of microspheres have been assessed by comparing the distributions of injections given sequentially, on the premise that these will be comparable in the absence of excessive embolisation. With 15μm microspheres, matching distributions have been found in the kidney, heart, spleen, skin, and gut after injections of up to 3 × 10⁵ microspheres in the rabbit (Warren and Ledingham 1974), and in femoral bone after injections of approximately 2 × 10⁶ in the dog (Gross *et al.* 1979, Jones *et al.* 1982) and 3.5 × 10⁵ in the rat (Schoutens *et al.* 1979). Clearly, such studies should be carried out in any proposed animal model.

The microsphere method is based on assumptions that are amenable to experimental testing, and allows measurement of bone blood flow without surgical interference. Before its use in any animal model, however, the constraints on the size of the microspheres and their number must be known.

1.2.5 QUANTITATIVE STUDIES OF BLOOD FLOW TO THE DIAPHYSIS

A compilation of the results from quantitative studies of the overall flow rate to the adult tibial diaphysis using the principal indicators is given in Table 1.2.1. There appear to be no figures available for the cortex alone as measured by the isotope clearance technique, but as the uptake of bone-seeking isotopes by the marrow is very low (Bosch 1969), the whole-bone flow rate given in the table will be close to cortical flow. There is general agreement that the rate of flow to the diaphyseal cortex is very low, of the order of 1 to 5 ml/100g.min. The marrow flow is low in the dog, in which it comprises mostly adipose tissue,

but higher in the rabbit where it remains more actively haematopoietic.

Exclusion studies using quantitative methods have been carried out in acute but not in chronic experimental models. Shim *et al.* (1968) measured strontium clearance by the femur in the rabbit during the first five minutes after ligation of the nutrient artery. The differences between the control and experimental limb gave the 'least rate' of supply by this vessel, as a part of its domain may have been supplied after ligation by other vessels. The flow rate to the diaphysis dropped by 71%. Tothill *et al.* (1987), using 15 ± 5 µm microspheres in dogs, found that occlusion of the tibial nutrient artery in three animals resulted in a drop of approximately 80% in the flow rate to the middle half of the tibia, or 66% allowing for the drop in flow to the control limb during the experiment. In studies in dogs using microspheres sized 8 to 15µm, Kunze *et al.* (1981) found a drop of 75% in the flow rate to the cortex of the tibial diaphysis after medullary reaming, which destroys the arterial and venous nutrient circulation and the anastomoses with the metaphyseal supply.

These results contrast with those of hydrogen washout studies. The flow rate to the middle third of the tibial cortex measured by hydrogen washout in the rabbit and dog was not affected by reaming of the medulla (Whiteside *et al.* 1978). Weiland and Berggren (1981), who claim that the routes of blood supply to the canine rib are similar to those of the diaphysis, found that blocking the medullary circulation by subtotal osteotomy and plugging with bone wax did not have a significant effect on the flow rate to the inner third of the cortex as measured by hydrogen washout. This disagreement as to the relative importance of the contribution of the nutrient circulation to the flow rate of the diaphysis may arise from differences in the volume of bone studied. Thus, while the clearance and microsphere studies give an average flow for whole segments

of cortex, the platinum electrodes of the hydrogen washout method probably reflect changes much more locally.

Removal of the diaphyseal periosteum appears to have less effect, as might be expected from the results of perfusion studies. Kunze *et al.* (1981) found cortical flow to drop only 31% after periosteal stripping, while Whiteside *et al.* (1978) found no effect. In both studies, combined stripping and reaming virtually abolished flow. Lifting of the periosteum will disrupt both arterial and venous channels, and no conclusions may be drawn from these studies as to the relative importance of either.

Presently, quantitative methods have done little to advance knowledge of the functional relationships of the components of diaphyseal blood supply. This arises from the difficulty in manipulating the network of vessels, and in particular the difficulty of controlling the arterial and venous channels independently.

Table 1.2.1   Previous estimates of blood flow to the adult tibial diaphysis. Flows are expressed as ml/min.100g

Animal	Method	Cortical flow	Marrow flow	Ref.
rabbit	H₂ washout	≈3.5 ± 0.1 (SD)	≈27–104†	1
"	"	≈4.1 ± 0.25 (SD)	–	2
"	"	≈2.5‡	–	3
dog	ms 8–15µm	1.02 ± 0.05 (SE)	–	4
"	" 15±5µm	3.0 ± 0.8 (SE)	–	5
"	" 15µm	1.96	–	6
"	" 15µm	2‡	7‡	7
"	" 15µm	3.7 ± 0.7 (SE)	–	8
"	" 15±5µm	1.0 ± 0.4 (SE)	–	9
"	" 15µm	2.1 – 4.7	–	10
"	" 15µm	–	7.4	11
"	" 15µm	4.89	–	12
"	" 15µm	1.6	–	13
"	Sr clearance	3.57 ± 0.68 (SE)	–	14
"	IAp washout	≈0.8	≈6.7	15

H₂ = hydrogen ms = microspheres Sr = strontium IAp = iodoantipyrine

Figures preceded by '≈' have been converted from units of ml/min.ml assuming the density of cortical bone to be 2 g/ml and that of diaphyseal marrow 1 g/ml

† Double component washout curves from upper diaphyseal marrow
‡ Figure read from histogram or diagram of results

Table 1.2.1 (cont.)   References

1   Whiteside, Lesker and Simmons 1977

2   Whiteside, Simmons and Lesker 1977

3   Whiteside *et al.* 1978

4   Kunze *et al.* 1981

5   Okubo *et al.* 1979

6   Richards and Schemitsch 1989

7   Schnitzer *et al.* 1982

8   Tøndevold and Eliasen 1982a

9   Tøndevold and Eliasen 1982b

10 Tothill, Hooper, McCarthy and Hughes 1987

11 Morris and Kelly 1980

12 Li *et al.* 1989

13 Tøndevold and Bülow 1984

14 Rand *et al.* 1981

15 McElfresh and Kelly 1974

1.3

THE CHANGES IN BLOOD FLOW TO THE DIAPHYSIS AFTER FRACTURE

The changes in blood flow after experimental fracture of a long bone have been investigated using the methods discussed in the previous section.

1.3.1 QUALITATIVE CHANGES IN VASCULARITY AFTER FRACTURE

Changes in the calibre of periosteal blood vessels occur almost immediately after fracture (Wray 1963), and an increase in vascularity as seen on microangiography is apparent within four days (Cavadias and Trueta 1965, Rhinelander 1968). While this change includes the medullary vessels, periosteal callus supplied by vessels from the periosteum and surrounding soft tissue is more prominent than endosteal callus in the first days, and this predominance of extraosseous supply to the callus continues until the medullary circulation is re-established across the fracture site (Rhinelander and Baragry 1962, Rhinelander 1968,1974, Rhinelander et al. 1968).

The timing of this change appears to depend on the stability of the fracture (Rhinelander 1974). Rigid fixation with a plate allows medullary vessels to cross the fracture within a week (Rhinelander 1968), while with a 'stable' but unfixed reduction this process takes three or more weeks (Rhinelander et al. 1968, Holden 1972, Chidgey et al. 1986). In the absence of fixation, it is the periosteal callus that supplies the stability required to allow the medullary circulation to regenerate.

Early movement at the fracture site appears to encourage the formation of cartilage in the vascular

external callus, which in other circumstances appears to
arise in areas of decreased vascularity (Rhinelander 1974,
Trueta 1974). Holden (1972) found that temporary devasc-
ularisation of the muscle around a double osteotomy of the
rabbit radius resulted in delay of the re-establishment of
the medullary circulation and an increase in the level of
cartilage formation. The appearance of a plate of cartilage
along the fracture line, surrounded by a rich vascular
network, presumably also results from excessive fracture
motion rather than from a deficiency in the blood supply
(Rhinelander 1968).

The height of microangiographic vascularity occurs at
about four weeks after fracture in the rabbit (Cavadias and
Trueta 1965, Brookes *et al.* 1970), at which time the
medullary circulation begins to take over the supply of the
external callus by branches running through the cortex
(Rhinelander and Baragry 1962, Rhinelander 1968, Chidgey *et
al.* 1986).

Internal fixation of the osteotomy has been noted to
affect vessel perfusion with contrast medium. In fractures
of the dog forelimb Rhinelander (1968) has observed that
the cortex under a tightly applied plate becomes devoid of
perfused vessels throughout its depth, presumably as a
result of blockage of the venous outflow, while an
intramedullary nail does not prevent vessel filling in the
outer third. The periosteal supply, or rather that arising
from the soft tissue, proliferates to compensate for any
damage to the medullary circulation from an intramedullary
nail or by interruption of the nutrient artery (Trueta and
Cavadias 1955, Göthman 1960b,1961, Cavadias and Trueta
1965, Oni 1987), and union occurs by external callus. The
other bone blood supplies are not so efficient in
supporting healing in the absence of this external supply.
Exclusion of the periosteum by a sheath was attempted by
Cavadias and Trueta (1965) after osteotomy of the rabbit
radius but only two adult animals did not sustain damage to
the nutrient artery. Endosteal callus appeared only in the
proximal fragment, and union was delayed with respect to

control fractures in both cases. These workers also found
that exclusion of both the periosteum and nutrient artery
results in delayed union unless both proximal and distal
nutrient vessels are filled via anastomoses between the
epiphyseo-metaphyseal supply and the nutrient circulation.
These findings were confirmed by Oni (1987).

1.3.2 QUANTITATIVE CHANGES IN BLOOD FLOW AFTER FRACTURE

After the observation that an osteotomy of the rabbit
humerus resulted in a doubling of blood flow to the
osteotomy site at four weeks when compared to the control
limb (Brookes *et al*. 1970, microsphere technique), a number
of quantitative studies have been carried out after
experimental fracture, most taking the form of the
comparison of one form of fixation with another.

Blood flow changes immediately after an osteotomy have
been measured in the dog femur using 8-15μm microspheres
(Kunze *et al*. 1981). Flow to the cortex dropped only
slightly proximal to the osteotomy, but distally flow
dropped from 1.61 ± 0.15 (SD) to 0.62 ± 0.08 ml/min.100g,
probably reflecting division of the nutrient circulation.
Internal fixation by plating or by screws further reduced
cortical flow. The standard deviations in this paper are
artificially low, as several cortical samples were taken
from each animal, and they do not all, therefore, represent
independent observations.

Without differentiating between bone proximal or distal
to an osteotomy, Smith *et al*. (1987) found flow to a 5cm
segment of tibial cortical bone in the dog measured with
microspheres (presumably of 15μm) to drop from 6.10 ±
0.30 (?SE) to 3.06 ± 0.66 ml/min/100g at ten minutes after
a transverse osteotomy through its centre. After four
hours, flow had dropped further to 1.77 ± 0.68, possibly as
a result of deterioration of the preparation during the
prolonged anaesthesia.

Blood flow during healing to a length of bone containing an osteotomy fixed by various methods has been studied in the dog by Kelly's group using strontium clearance, iodo-antipyrine washout, and the microsphere method (Laurnen and Kelly 1969, Paradis and Kelly 1975, Barron *et al.* 1977, Hughes *et al.* 1979, Rand *et al.* 1981, Lewallen *et al.* 1984, Wu *et al.* 1984, Hart *et al.* 1985, Nutton *et al.* 1985, Smith *et al.* 1987). The results of these studies are summarised in Tables 1.3.1 and 1.3.2, with the addition of the one other similar study, performed by Chidgey *et al.* (1986).

The only such study on osteotomies not treated by internal or external fixation comprises a control group subjected to osteotomy of the ulna (Barron *et al.* 1977, Table ref 1). Strontium clearance of the fracture site in this group rose from 3.88 ± 1.50 (SE) ml/min.100g at one day to a peak of 30.00 ± 7.18 at fourteen days. At forty-two days clearance was still raised.

From the remaining series it is apparent that surgical fixation of the fragments is associated with changes in the subsequent levels of bone blood flow. Smith *et al.* (1987, ref 6) found flow to the cortex of the tibial shaft over the first two weeks to be statistically significantly lower in those osteotomies treated by intramedullary nailing compared to those held by a plate or by external fixation. Plating was found to reduce flow to the underlying cortex, but not to the same degree as caused by nailing. Barron *et al.* (1977, ref 1) found lower strontium clearances after nailing in the ulna, which appeared to be largely due to rises in flow to plated osteotomies greater than in those left unfixed, with flow to nailed osteotomy sites remaining insignificantly different from the controls. However, Rand *et al.* (1981, ref 2) noted clearances to nailed tibiae to be significantly higher than those to plated bones at forty-two and ninety days. In general, flow appears to peak between seven and twenty-one days after osteotomy, which is earlier than might be expected from perfusion experiments.

Evidence for recruitment of soft tissue blood supply to the cortex after disruption of the nutrient circulation by an osteotomy has been found two weeks after plated osteotomies of the tibia in dogs (Strachan *et al.* 1990). Ligation of the nutrient artery at this time resulted in no drop in cortical flow, while marrow flow was reduced by more than half. This contrasts to the large reductions in diaphyseal cortical flow seen after nutrient artery ligation in intact bones in this study and others (Shim *et al.* 1968, Tothill, Hooper, McCarthy and Hughes 1987), and emphasises the importance of the periosteal and soft tissue supply to the cortex during the early period of fracture healing.

After an osteotomy, flow to the bone as a whole is found to increase in addition to that to the fracture site (Laurnen and Kelly 1969, Paradis and Kelly 1975, Barron *et al.* 1977, Rand *et al.* 1981). Counting of serial sections of bone for microspheres injected into the tibial nutrient artery of dogs ten days after an osteotomy (Lavender *et al.* 1979) suggests that the changes in flow are greater near the osteotomy but are also found throughout the length of the tibial shaft. While perfusion studies show that the contralateral intact bone shows increases in vessel filling (Rhinelander 1968, Brookes *et al.* 1970), there appears to be no consistent variation from normal strontium clearances in the control tibia of dogs (Laurnen and Kelly 1969, Paradis and Kelly 1975), while with the microsphere technique the control flows "changed little" after the creation of a tibial diaphyseal cortical defect (Nutton *et al.* 1985). One consequence of a generalised increase in bone blood flow would be that any differences between control and fractured limbs will be reduced, but at present there is no strong evidence for this.

These quantitative studies share several features in common. Firstly, blood flow has been measured at the fracture site as a whole, without differentiating between the proximal and distal fragments. Secondly, except for one part of one study, some form of internal or external

fixation has been used in all, although the effects of exclusion of the marrow and soft tissues by implants has yet to be adequately defined. Thirdly, most have relied on the techniques of indicator clearance or washout which are subject to several major disadvantages (Sections 1.2.2 and 1.2.3). Finally, they have all been performed after surgical osteotomies rather than after a fracture produced by external trauma, and no effort has been made to assess the effect of different degrees of blunt trauma on subsequent bone blood flow. The present study aims to extend the quantitative study of tibial shaft blood flow after fracture in these four areas.

Table 1.3.1   Mean estimates of bone blood flow at the fracture site in the first two weeks after osteotomy in the dog. Flows are expressed as ml/min.100g.

bone/method			1 day	2 days	7 days	14 days	ref
ulna	Sr	control	3.88	-	-	30.00	1
		rod	1.74	-	-	24.26	
		plate	1.72	-	-	28.10	
tibia	Sr	rod	0.79	-	-	20.23	2
		plate	1.11	-	-	23.12	
tibia	Sr†	plate	4.7	-	6.1	19.4	3
tibia	Sr	plate	-	-	3.03‡	-	4
tibia	IAp	plate	-	-	3.21‡	-	4
tibia	IAp*	plate	-	-	-	6.65	5
tibia	ms	rod	-	1.52	-	14.09	6
		plate	-	2.87	-	17.05	
		EF	-	2.60	-	19.51	
tibia	ms*	defect	-	-	25.10	25.43	7

Sr = strontium clearance, marrow and cortex

IAp = cortical flow by iodo-antipyrine washout

ms = cortical flow by the microsphere method (15µm)

control = no fixation

rod = intramedullary nail fixation

plate = plate internal fixation

EF = external fixation

defect = cortical defect, not osteotomy

† = flow to whole bone, read from graph of data

‡ = flow at five days

* = flow to cortex and marrow combined

References (ref) are given in Table 1.3.3

Table 1.3.2 Mean estimates of bone blood flow at the fracture site beyond two weeks after osteotomy in the dog. Flows are expressed as ml/min.100g.

bone/method			21 days	42 days	90 days	120 days	ref
ulna	Sr	control	-	15.12	-	-	1
		rod	-	19.61	2.26	-	
		plate	-	24.44	5.20	-	
tibia	Sr	rod	-	19.72	14.31	7.94	2
		plate	-	13.20	5.65	5.92	
tibia	Sr†	plate	-	11.3	4.8	-	3
tibia	Sr	EF:					
axial compression			-	-	11.96	-	8
no compression			-	-	13.11	-	
tibia	Sr	EF:					
		4-pin	-	-	-	12.7	9
		6-pin	-	-	-	10.2	
tibia	Sr	plate	-	-	-	10.0	10
		EF	-	-	-	13.2	
tibia	Sr	plate	6.77	4.96	1.63	-	4
tibia	IAp	plate	3.59	2.82	2.80‡	-	4
tibia	IAp*	plate	-	-	4.77‡	-	5
radius	ms	pinned:					
		cortex	36.05	6.18	-	-	11
		marrow	67.70	14.48	-	-	

Key as for Table 1.2

pinned = fixation by transfixion to ulna

† = flow to whole bone, read from graph of data

‡ = flow at eighty-four days

* = flow to cortex and marrow combined

References (ref) are given in Table 1.3.3

Table 1.3.3   References for Tables 1.3.1 and 1.3.2

1   Barron *et al.* 1977

2   Rand *et al.* 1981

3   Laurnen and Kelly 1969

4   Paradis and Kelly 1975

5   Hughes *et al.* 1979

6   Smith *et al.* 1987

7   Nutton *et al.* 1985

8   Hart *et al.* 1985

9   Wu *et al.* 1984

10   Lewallen *et al.* 1984

11   Chidgey *et al.* 1986

The figures from Paradis and Kelly (1975) have been calculated from the individual data given, using the fracture-site specific gravity of 1.48 found by the authors.

The figures from Chidgey *et al.* (1986) have been calculated from their figures for 1cm segments either side of the osteotomy.

1.4

SUMMARY AND OBJECTIVES OF THE STUDY

Delayed union of fractures of the tibial shaft is a significant cause of morbidity in the young adults who commonly sustain this injury. The definition of union is at present imprecise, and the factors associated with its delay may be assessed most appropriately from clinical series of fractures treated without internal fixation. These factors are found in turn to be associated with the degree of the force causing the fracture. The underlying mechanism of this effect may be the increased vascular damage and subsequently retarded vascular response that might be expected in higher energy injuries.

However, the measurement of bone blood flow presents severe difficulties in the clinical setting. In animals, qualitative models both of bone grafting and of vascular damage in combination with fracture have given support to the hypothesis that the rate of union is related to the available blood supply, but no quantitative study has assessed either fractures caused by blunt trauma or the effects of damage to the principle routes of blood supply.

The measurement of bone blood flow is complicated by the diffuse arterial supply and venous drainage of this tissue. Of the methods available, those that rely on the exchange between bone and blood of an indicator suffer several disadvantages that are overcome by the radioactive microsphere method.

The aims of the present study were therefore :

1. To develop a technique to measure tibial diaphyseal blood flow in an animal model using the radioactive microsphere method.

2. To quantify the effects of the exclusion of the marrow or soft tissue supplies on the blood flow to the proximal and distal fragments of an experimental fracture of the tibial diaphysis.

3. To quantify the changes in blood flow to the proximal and distal tibial diaphysis during the healing of fractures caused by blunt trauma of differing forces.

VALIDATION OF ANIMAL MODEL

2.1

TECHNICAL CONSIDERATIONS

2.1.1 EXPERIMENTAL ANIMALS

2.1.1.1 *Choice of experimental animal*

The ideal animal for quantitative studies of tibial shaft blood flow during fracture healing would have hindlimbs sufficiently large to enable the reproducible use of blunt trauma to produce fractures near a specified site along the bone. Further, it should be moderate in cost. This second requirement excluded the use of the dog, and while the rat is inexpensive it was considered too small. Between these extremes is the rabbit, which has been extensively used in perfusion studies of changes in vascularity during immobilisation and fracture healing (Trueta and Cavadias 1955, Geiser and Trueta 1958, Hulth and Olerud 1960a,b, Göthman 1960a,b, 1962, Ferguson and Akahoshi 1960, Trueta 1963, Trueta and Cavadias 1964, Mohanti and Mahakul 1983, Oni 1987, Oni *et al*. 1989).

The rabbit tibiofibula differs from that of man by the fusion of the distal parts of the bones during growth. As the majority of clinical tibial shaft fractures are accompanied by a fracture of the fibula, the absence of a separate bone is not a significant disadvantage. It is also easier to obtain reproducible fractures by blunt trauma when only one bone is to be fractured.

Little information is available as to the comparability of the left and right tibiofibulae. Mechanically, left and right rabbit tibiofibulae appear to respond similarly to torsion (White *et al*. 1974), although they were tested in opposite directions. It has been claimed that one bone is usually heavier than the other and that this difference is

statistically significant, but the side of this 'dominance' varies (Singh 1971). Critically, the age of the animals used in this experiment was not stated. The blood flow to left and right femurs of the rabbit have been found not to differ significantly using the microsphere method (Syftes-tad and Boelkins 1980, n=6), but there do not appear to have been any such studies of the tibiofibula. In the present study, the experimental limb was selected randomly whenever possible to minimise the effects of any left to right differences.

There have been no reports of microsphere estimation of *absolute* blood flow to the normal rabbit tibia. Microsphere estimations of flow to the rabbit femur have been made by Lunde and Michelson (1970) and by Syftestad and Boelkins (1980). The former workers, using microspheres sized 15 ± 5 μm (SD), found the flow to the diaphyseal cortex to be 1·0 ± 0.7 (SD) ml/min.100g, and to the diaphyseal marrow 25·0 ± 14.6 ml/min.100g. The latter workers, also using microspheres with an average diameter of 15μm, give a figure of 29.7 ± 5.7 (SD) for the diaphyseal marrow. These results are similar to those obtained in the rabbit tibia using hydrogen washout, and in the dog using a variety of methods (Table 1.2.1, Section 1.2). There appear to be no fundamental differences between the two species with regard to diaphyseal cortical blood flow, while the differences in marrow flow probably result from differences in haematopoietic activity.

2.1.1.2 *Maturity of the experimental animals*

There is evidence that tibial blood flow drops upon skeletal maturity and with increasing age, and that this change affects diaphyseal bone as well as bone near the growth plates. Schnitzer *et al.* (1982) have measured tibial diaphyseal cortical flow in conscious dogs using the microsphere technique. In immature animals aged four months, flow was 6 ml/min.100g, while in skeletally mature animals it had reduced to 2 ml/min.100g. In the rabbit, upper tibial metaphyseal flow was found to drop

progressively with age over at least the first thirty months (Kita *et al.* 1987, using hydrogen washout). The proportion of cardiac output to the rat tibiofibula, as estimated by the relative distribution of 15μm microspheres, declines over the age range of two to seventeen months (MacPherson and Tothill 1978).

Studies of growth and tibial epiphyseal closure in the New Zealand White rabbit (Masoud *et al.* 1986) have shown that in females, the sex used in this study, the distal growth plate fuses radiologically at 14 to 20 weeks (mean 17 weeks), whereas the proximal plate fuses later at 22 to 30 weeks (mean 25 weeks). Beyond 22 weeks of age, any further growth of the tibia is very slight, at less than 0.5 mm per fortnight. This age corresponds with a body weight of 3.5kg. As far as possible, animals above this weight were used in the following studies, although, as some animals lost weight in the first few days after delivery from the supplier, lighter animals were not necessarily immature.

2.1.1.3 *Animal husbandry*

The details below are given according to the recommendations of the Working Committee for the Biological Characterization of Laboratory Animals (1985).

Adult female New Zealand White rabbits of conventional microbiological status were obtained from Shrubacre Rabbits (Beccles, Suffolk). Before recovery experiments the animals were acclimatised for at least 2 weeks in free-range rooms.

Room temperature was maintained at 20 to 22° centigrade with a relative humidity of 50 to 65%. Lighting was artificial, from fluorescent tubes, and turned on from 8am to 8pm each day. Diet comprised *Rabma* pellets (SDS, Witham, Essex) supplemented with green vegetables and carrots, with tap-water *ad libitum*. Bedding was provided by *Beta-Bed* wood-chip litter (Datesand Ltd, Manchester).

2.1.2 CATHETERISATION OF THE RABBIT

2.1.2.1 *Anaesthesia*

The method of anaesthesia selected by the staff of the Biomedical Services Unit comprised premedication with intravenous midazolam, induction with intramuscular *Hypnorm* (fentanyl and fluanisone) and maintenance with halothane breathed spontaneously. The use of spontaneous ventilation avoided the use of either neuromuscular blocking agents or the deep plane of anaesthesia necessary to allow positive pressure ventilation, and also avoided the requirement for tracheal intubation which is difficult in the rabbit. A tracheotomy was not considered suitable for experiments with recovery.

The induction agents used in high doses without halothane give a period of surgical anaesthesia in the rabbit of approximately 100 minutes (Flecknell and Mitchell 1984). At this dosage there is no significant effect on arterial blood pressure or heart rate, but the *Hypnorm* results in marked respiratory depression associated with a drop in arterial oxygen tension to approximately 6.5KPa or 45mmHg (Flecknell *et al.* 1989). In the present study, the use of halothane enabled the dosage of *Hypnorm* to be reduced to approximately one third of that otherwise required.

Wyler (1974) has studied the changes in cardiac output and organ flows in rabbits anaesthetised with halothane alone. Cardiac output dropped to 63% of control values, and mean arterial pressure was reduced to 75%. The organ flows tended to decrease in line with the changes in cardiac output, with no changes in their vascular resistance, *ie* there was no unphysiological redistribution of blood flow despite the fact that measurements were taken after a minimum anaesthetic time of almost two hours. This lack of a major sympathetic outflow response differentiates the hypotension of halothane anaesthesia from the effects of haemorrhage. The stability of the peripheral vascular resistances indicates that this agent has little effect on

arteriovenous anastomoses, which contrasts with the effects of the commonly used barbiturate pentobarbitone (Kazmers *et al.* 1984).

Wear *et al.* (1982) have shown that the baroreceptor reflexes are still present but blunted in rabbits given different multiples of the mean inspired anaesthetic concentration of halothane. Increasing halothane concentrations resulted in an increasing delay before the heart rate response to rises in arterial pressure produced by phenylephrine, and the response was also slowed.

The predominantly central location of the effects of halothane make it a suitable anaesthetic for the study of the peripheral circulation.

2.1.2.2 *Catheter sites*

While injection of microspheres into the distal aorta provides adequate mixing to the hindlimbs (Gregg and Walder 1980) and reduces the number of microspheres necessary in the injectate, in the rabbit this technique requires a laparotomy if catheterisation via a hindlimb vessel, with consequent interference with the flow to that limb, is to be avoided. Catheterisation from the neck is prevented by the anatomical arrangement of the aortic arch, which lies virtually in the sagittal plane with the origins of the carotid vessels lying anteriorly. Thus a catheter introduced retrogradely into either carotid artery will always enter the ascending aorta and left ventricle. As a major surgical procedure such as laparotomy is prejudicial to the maintenance of an undisturbed peripheral circulation, a central site for microsphere injection was selected.

The three possible central sites are the left atrium, the left ventricle, and the ascending aorta. In the absence of facilities for a trans-septal approach (Simkin *et al.* 1990), left atrial injection requires a thoracotomy, and while this can be carried out at a time remote from the injection of the microspheres if the catheter is implanted chronically (Warren and Ledingham 1972), this offers little

advantage if in any event catheterisation of a peripheral artery is required for the taking of withdrawal samples.

The left ventricular site provides adequate mixing as assessed by comparing flows obtained from paired hindlimb bones in the dog (Jones *et al.* 1982, Nutton *et al.* 1984, Riggs *et al.* 1984, Li *et al.* 1989), and is easily accomplished by retrograde catheterisation of either common carotid artery in the rabbit. However, it precludes the monitoring of arterial pressure during withdrawal sampling unless a third catheter is introduced. Positioning the injection catheter in the ascending aorta overcomes this problem, and also removes the requirement for warming the injectate to prevent ventricular dysrhythmias (Heymann *et al.* 1977).

The potential problems with aortic injection are uneven distribution of microspheres to the major vessels, and streaming of the microspheres within the blood distributed, giving rise to inaccuracies of measurements within organs. Such problems can be investigated by comparing the counts in paired organs, and by comparing counts in blood samples taken simultaneously at different sites.

In the rabbit, Aalto and Slätis (1984) give figures for tibial counts after injection into the ascending aorta of 15 ± 3 μm microspheres. These show no statistically significant difference on the paired t test (n=4). Injection of 15μm microspheres into the thoracic aorta via the left brachial artery was found to give less than 2% variation between tibial counts in two animals (Court-Brown 1985), while injection just above the aortic bifurcation gave femoral counts not differing significantly on the paired t test for either 15μm or 50μm microspheres (Gregg and Walder 1980, n=10).

The difference in counts between blood samples taken from the saphenous arteries of opposite hindlimbs after injection of 11.1 ± 0.3 μm microspheres into the abdominal aorta was 5.9 ± 4.8 (SD) % (Wootton and Doré 1986), although no statistical analysis was carried out, and the small number of microspheres injected (5×10^5) is likely

to result in large random variation. Lunde and Michelson (1970) found the variation between samples taken from two branches of the same posterior tibial artery to remain under 10% after abdominal aortic injection of 15 ± 5 μm microspheres. The number of microspheres injected was not stated.

Thus the current evidence is that aortic injection in the rabbit gives mixing of microspheres of 15μm or more in size adequate to allow comparison of estimated flows in paired bones of the hindlimb. As smaller microspheres are more evenly distributed across arterial lumens (Phibbs and Dong 1970) it is probable that their mixing will be better rather than worse.

Catheterisation through a common carotid artery necessarily results in occlusion of that vessel, with a consequent drop in the pressure acting on the carotid sinus baroreceptors. Edwards *et al.* (1959) studied the effects of carotid occlusion in conscious rabbits, and found that unilateral occlusion led to a rise of some 10mmHg in mean systemic arterial pressure, but no change in cardiac output as measured by dye dilution. Occlusion of both carotid arteries led to a rise in mean pressure of approximately 20mmHg, but again no change in cardiac output was observed. These rises in arterial pressure had not been seen in anaesthetised animals, and it appears that the collateral circulation of the rabbit is sufficient to obviate any significant circulatory effects of carotid occlusion.

The brachial artery was selected as the site for the withdrawal catheter, as it is readily accessible without a major surgical exposure, and it enables sampling from a vessel not involved in supplying blood to the lower limbs. It has been shown that virtually all microspheres have passed through the brachial artery of the rabbit by one minute after an injection made over 30 seconds into the left atrium (Thomas 1983).

2.1.2.3 *Catheter type*

The catheters chosen for this study were 16G epidural catheters, supplied by Portex of Hythe. These catheters are fine enough for ready cannulation of the brachial artery of the rabbit, their rounded ends assuring easy passage. Each catheter has three side orifices, directed at 120° to each other, and thus injected microspheres are distributed more evenly across the width of the aorta than with a single outlet. An adaptor to allow direct connection to a standard syringe or three-way tap is supplied with each catheter.

2.1.2.4 *Withdrawal pump calibration*

The rate of withdrawal of the blood sample during and after microsphere injection must be known in order to allow calculation of absolute blood flows. The most accurate method is probably to weigh the syringe before and after sampling, with division of the difference by the time and the specific gravity of whole blood, but a sufficiently accurate balance was not available at the Biomedical Services Unit. This was not a significant disadvantage, as the pump used was found to give reproducible rates of withdrawal, and by calibrating the pump the precise timing of the sampling was not necessary.

The pump was of type 871104, supplied by B Braun of West Germany. The rate of withdrawal was selected to approximate 2·5 ml/min, being less than 1% of the cardiac output of an adult rabbit (Edwards *et al.* 1959, Cumming and Nutt 1962, Syftestad and Boelkins 1980), and thus small enough not significantly to interfere with the circulating volume while being large enough to reduce proportionate variation in withdrawal rate.

Calibration was carried out during access to an accurate balance (type OB152, Oertling, Birmingham). The 20ml syringe to be used for the measurement of blood flow was weighed to 0.01g after drawing up approximately 1ml of water, the volume of heparinised saline used for the animal experiments, and mounted on the pump. Water was withdrawn

from a container through a withdrawal catheter for a two minute period. The syringe was then disconnected and reweighed. The difference in weight divided by two gave the withdrawal rate in ml/min. This process was carried out ten times and the average rate calculated. It was found that the coefficient of variation remained less than 1%.

2.1.3 RADIOACTIVE MICROSPHERES

2.1.3.1 *Microsphere type*

For the measurement of blood flows over periods of several days or weeks, the microspheres must be non-biodegradable and must retain their label. Further, to enable accurate flow measurement they must be sized as closely around the stated mean as possible (Section 1.2.4). The *Nen-Trac* microspheres supplied by DuPont and used in this study are manufactured from styrene-divinyl benzene ion-exchange resin, which is not metabolised, and the size distribution of most lots has a standard deviation of only 0.1μm. The microspheres are coated with a polymeric resin to reduce aggregation and isotope leaching. The company were unable to supply information as to the degree of leaching *in vivo*, and therefore a pilot study was undertaken to determine this (Section 2.5).

The specific gravity of the microspheres is approximately 1.4. Microspheres varying in specific gravity from 1.1 to 1.6 show no tendency to gravitate to the lower half of the lumen of arteries of 0.7 to 1.75mm in internal diameter (Phibbs and Dong 1970).

The microspheres were supplied suspended in physiological saline, with 0.01% *Tween 80* detergent added to reduce further any aggregation.

2.1.3.2 *Choice of isotopes*

The factors considered in the choice of labelling isotopes were half-life, emission energy spectrum, availability, and cost.

A long *half-life* is desirable for the study of bone blood flow during fracture healing, as this process takes place over a period of weeks. A long half-life also confers the advantage of a long shelf life both for the stock suspension of microspheres and for the experimental samples. Further, any corrections for delays between different counts are reduced in magnitude and accuracy made easier to maintain.

The *spectral distributions* of the emission energies of different isotopes must be sufficiently widely separated to be distinguishable within the same experimental sample. To separate the spectra, each energy peak must be clear of any interference from the next less energetic isotope (Heymann *et al.* 1977). This practice of spectral 'stripping' is discussed in Appendix 1.

The *magnitude* of emission energy must be sufficient to prevent significant quenching by the tissue of the experimental samples. However, it has been found that the radiation of even very weak γ-emitters, such as iodine-125 with a peak energy of 35 KeV, is not attenuated by cortical bone (Gross *et al.* 1979, Tøndevold and Eliasen 1982b). While Morris and Kelly (1980) dispute this, they provide no direct evidence.

The commercial *availability* of different labels for microspheres is limited to about twelve (as supplied by DuPont and the 3M Company), ranging from iodine-125 with an emission energy peak of 35 KeV to scandium-46 with peaks of 889 and 1120 KeV. Over this range the *cost* of the more expensive labels is approximately 40% above that of the cheapest. Further, the labels are not universally available across the whole range of microsphere sizes.

For this study gadolinium-153 (Gd[153]) and tin-113 (Sn[113]) were selected for their long half-lives, having

shelf-lives sufficiently long to offset the initially higher cost. Samples of the stock suspensions were counted on the two-channel counter to be used for the subsequent experiments (Type 80000 Gamma Sample Counter, LKB Instruments Ltd, UK), and the energy windows of the counter adjusted to cover the respective energy peaks of 97-103 KeV and 393 KeV. While the former 'region of interest' contained counts from both isotopes, the latter was adjusted to exclude any counts from the less energetic Gd^{153}. This enabled subsequent stripping of experimental counts, in order to distinguish the two isotopes (Appendix 1).

2.1.3.3 *Microsphere dispensing*

As they are not in solution, it is impossible to dispense a precise number of microspheres from the stock suspensions. The number drawn up into a syringe for injection can be found by counting the syringe in a gamma counter, but no counter was available at the Biomedical Services Unit where the animal studies were carried out. An alternative is to deliver a sample into a vial, counting the sample both before and after as many microspheres as possible have been drawn into the syringe at the Unit, with the syringe washed out into the vial after the experiment to retrieve any microspheres not injected. However, this is both time-consuming and wasteful of an expensive commodity, as it is not possible to draw up into a syringe every one of the microspheres from a vial.

With the reference sample technique, the precise number of microspheres injected does not need to be known, and therefore they were dispensed by volume. From the information supplied with each batch by the manufacturer, the approximate number of microspheres contained in 1ml of the stock suspension could be calculated, and to obtain an approximate number of microspheres in an injectate, the required volume was drawn up after agitation of the

suspension to ensure even distribution of the microspheres in the saline.

2.1.3.4 *Microsphere disposal*

Almost all the microspheres used in this study came to lie in the carcases of the experimental animals, and these are disposed of by burning in an incinerator. With the exception of iodine, the isotopes used as labels for microspheres are those of heavy metals, with the result that most of the radioactivity remains in the ash rather than being dispersed into the atmosphere (Brekke *et al.* 1985). The limits imposed on the disposal of these isotopes are thus stringent.

During the course of this work, the manufacturer discontinued without warning the supply of low-activity microspheres (3-7 mCi/g), and subsequently only high-activity batches could be used (7-15 mCi/g). This effectively cut by half the number of injections that could be carried out, and therefore for most of the recovery experiments only one measurement of blood flow was obtained in each animal.

2.2

SIMULTANEOUS MEASUREMENT OF BLOOD FLOW TO THE TIBIAL DIAPHYSIS OF THE ADULT RABBIT USING MICROSPHERES LABELLED WITH TWO DIFFERENT RADIO-ISOTOPES

2.2.1 INTRODUCTION

Before the use of the rabbit tibia as an experimental model it is necessary to investigate the appropriate size and number of microspheres to be injected for the measurement of bone blood flow (Section 1.2.4). These requirements in turn depend on an injection technique that gives reliable mixing of the microspheres, and also on a counting technique that gives reliable separation of two isotopes, so that two microsphere injectates can be compared directly in the same animal. Subsequently, the technique can then be used to obtain two measurements of flow in the same animal at different times.

An assessment both of mixing and of isotope separation can be obtained by the simultaneous injection of microspheres of the same size distribution but labelled with the two different isotopes. This allows paired analysis of the flows to matching samples in the two hindlimbs to investigate mixing, and of the flows to each tissue sample calculated with the two isotopes to assess separation. If mixing and separation are accurate, there should be no differences between the paired flows.

2.2.2 METHOD

The principles of the anaesthesia, catheter placements, catheter type, withdrawal pump calibration and choice of

microspheres used in this study have been discussed in Section 2.1.

2.2.2.1 *Animal preparation*

The study was performed on 12 adult female New Zealand White rabbits weighing 3.00 - 4.25 kg. General anaesthesia was induced with 5·0 mg of midazolam (Roche, Welwyn Garden City) via an ear vein and 0.3 ml of intramuscular *Hypnorm* (fentanyl 0·315 mg/ml with fluanisone 10 mg/ml, Janssen Pharmaceutical Ltd., Oxford), and maintained with halothane administered via a snout mask. The animals were positioned supine on a bead-bag moulded to maintain the neck in extension, this position being maintained by evacuating air from the bag. The electrocardiogram was monitored by cutaneous needle electrodes connected to a *Diascope DS521* monitor (Simonsen and Weel, Denmark).

After shaving the front of the neck and the medial aspect of the left forelimb, the left common carotid artery was exposed through a longitudinal neck incision. Two ligatures were passed beneath the artery, the cranial ligature tied and a bulldog clip applied caudally. The artery was opened with fine scissors and a 16 gauge epidural catheter (Portex, Hythe) filled with heparinised 0.9% saline (20 units/ml) introduced, and passed caudally into the left ventricle. The arterial pressure was monitored by connecting the catheter to a pressure transducer (Model 800, Bentley Laboratories, Uden, Holland) via a three-way stopcock that allowed injection through the catheter via its side port. The pressure waveform and the mean pressure were displayed on a Type 128A monitor (Kontron Medical, Watford). The catheter was withdrawn until the pressure wave-form indicated that its side-ports lay just above the aortic valve, allowing monitoring of mean arterial pressure, and secured in place. If the aortic catheter could not be passed into the ventricle, it was left at the position where the resistance of the aortic valve could be clearly felt. Post mortem studies showed

that this procedure also reliably situated the side-ports of the catheter in the ascending aorta.

The left brachial artery was cannulated with a similar catheter which was attached to a calibrated withdrawal pump (Type 871104, B Braun, West Germany) set to 2.529 ± 0.006 (SE) ml/min for the first eight experiments. For the last four experiments a new withdrawal syringe was used calibrated at 2.634 ± 0.007 (SE) ml/min.

2.2.2.2 *Microsphere injection*

A mixture of approximately $5 - 6 \times 10^6$ microspheres sized 11.3 ± 0.1 (SD) μm and labelled with Gd^{153} and the same number of microspheres of the same size distribution labelled with Sn^{113} was drawn up into an insulin syringe. The mixture was placed on a vortex mixer for 30 seconds to distribute the spheres evenly, and the syringe agitated by hand until the spheres were injected.

The withdrawal pump was started and approximately 30 seconds later the mixture of microspheres was injected via the aortic catheter, followed by a flush of heparinised saline. The total injection period, including the flush, was approximately 30 seconds. The withdrawal sampling was continued for a further 2 minutes.

The animals were then killed by an overdose of pento-barbitone, and the tibiae excised together with their anterior musculature. The blood sample was emptied into counting tubes, and the withdrawal syringe rinsed into the tubes with heparinised water, both to clear it of micro-spheres and to lyse the blood cells. Lysis was facilitated by the addition of ammonium hydroxide solution, sufficient to give a final concentration of approximately 1% (w/v).

2.2.2.3 *Preparation of bone and muscle samples*

The tibiae were stored in a cold room at 3 ± 1 °C, and prepared within 72 hours of excision.

A combined sample of the tibialis anterior and extensor digitorum muscles, which are closely apposed, was obtained by removing the upper part of the muscles containing the neurovascular pedicle and the lower part of the muscles containing the tendons, together with any remaining deep fascia, to leave a middle section comprising muscle tissue only.

The proximal ends of the bones above a point 1cm distal to the lower end of the tibial tuberosity were removed with a 1mm hacksaw, together with the distal ends below a point 1cm proximal to the medial malleolus. The fibula was removed at the distal tibiofibular junction.

The length of shaft obtained was cleared with a scalpel of all soft tissue, including the periosteum, and sawn transversely into halves. The marrow from each half was evacuated by hand. The resulting cortical samples were washed using a jet of tapwater to remove any remaining extracortical microspheres. All samples were placed in pre-weighed counting tubes and the tubes re-weighed to 0·01g using *OB152* electronic scales (Oertling, Birmingham).

The soft tissue samples were difficult to place evenly in the bottom of a counting tube, and therefore they were centrifuged at 3000 rpm for 10 minutes. Their heights within the tubes were then measured. The heights of the cortical samples were also recorded.

Before counting, a drop of *Decon* detergent (Decon Laboratories Ltd, Hove) was added to each of the tubes containing blood from the withdrawal sample, in order to reduce adherence of the microspheres to the sides of the tubes. They were then centrifuged at 3000 rpm for 15 minutes to bring the microspheres down to the zero reference height.

2.2.2.4 *Counting of samples*

All samples were counted with the 80000 γ-counter (LKB Instruments Ltd., Selsdon) with windows set to maximise

counts at the Gd[153] and Sn[113] energy peaks of 97-103 and 392 keV respectively. At least 1×10^4 counts were obtained for each sample in each channel, to reduce the coefficient of variation to 1%. Background counts for each channel were obtained similarly.

2.2.2.5 *Analysis of results*

Counts were 'stripped' with correction for specimen height, as described in Appendix 1. The heights of the blood samples were taken to be zero.

Blood flow was calculated by equation 1.16 :

$$F_s = (C_s / C_{ref}).F_{ref}$$

where F_s = specimen flow
   C_s = specimen count
   C_{ref} = reference sample count
   F_{ref} = reference sample flow

All flows were expressed as ml/min.100g. The number of microspheres in each sample was calculated by dividing the count for each isotope by the specific activity per microsphere, after correction for decay (Appendix 1).

The blood flows obtained with the two microsphere labels were subjected to Student's paired *t* test, and linear regression analysis was performed to express the flows obtained with the Sn[113] label as a function of those obtained with the Gd[153] label.

2.2.3 RESULTS

The mean flows obtained for the different tissue samples are given in Table 2.2.1. Tables of the full data are given in Appendix 3, Tables A.3.1 to A.3.3.

2.2.3.1 *Animal preparation*

The microsphere injections were administered at 33 – 45 minutes from induction of anaesthesia (mean 39.7 ± 1.50 (SE)). Heart rate at the time of injection varied from 182 to 283 beats per minute (mean 226.7 ± 7.68 (SE)), and mean arterial pressure from 45 to 84 mmHg (mean 59.9 ± 3.58 (SE)). In no animal did the injection of the microspheres cause any detectable disturbance of these parameters.

2.2.3.2 *Microsphere mixing*

There were no statistically significant differences between the flows obtained in paired left and right samples, either with the microspheres labelled with Gd^{153} or with Sn^{113}. Thus the microspheres appeared to be evenly distributed to the hindlimbs.

2.2.3.3 *Microsphere numbers*

The numbers of microspheres lodged in each sample varied widely (Table 2.2.2). While over 92% of muscle and proximal marrow samples contained at least 150 microspheres of each isotope, with the cortex and distal marrow samples this proportion was 50% and 63% respectively. Table 2.2.3 gives the results of combining the flows to the samples of the different tissues to give composite samples containing at least 150 microspheres.

2.2.3.4 *Separation of the two isotopes*

The differences between the flows obtained simultaneously with the two labels were not significantly different in the marrow and muscle samples, while the flows obtained with the Sn^{113} label were significantly greater in the cortical samples. With all samples, regression analysis gave very high correlation coefficients (Table 2.2.4) and high degrees of statistical significance of correlation, but with the cortical samples the regression coefficients, or

slopes, were not as close to unity as with the soft tissue samples.

2.2.4 PRELIMINARY DISCUSSION

This study has shown that the present technique gives even microsphere mixing, with no marked perturbation to the circulation after microsphere injection. The isotopes were satisfactorily separated in the marrow and muscle samples. The proximal marrow in the rabbit is macroscopically red, and thus this part of the marrow receives two to three times the blood flow of the distal marrow, which is predominantly adipose. Two problems were, however, identified.

Firstly, the low flows to the cortex and distal marrow, and the small amounts of the latter, resulted in many of these samples containing small numbers of microspheres. When smaller numbers of microspheres reach a tissue, random variation in their numbers increases, and thus the calculated flow rates are more variable (Katz et al. 1971, Buckberg et al. 1971). It is thus more difficult to demonstrate differences between two groups of flows. The flow to each tibia as a whole in the rabbit accounts for approximately 0.12% of cardiac output (Tothill and MacPherson 1986), and thus a dose of 5×10^6 microspheres would be expected to result in approximately 6,000 microspheres lodging in each tibia. The average numbers found experimentally in the diaphysis were 1484 and 1599 for the microspheres labelled with Gd[153] and Sn[113] respectively. These are less than might be expected, and this, together with the wide variability in microsphere numbers, demonstrates the difficulty of dispensing and delivering a stated dose (Section 2.1.3.3).

As the number of microspheres injected is large, and the probability of any sphere entering the tibia is small, the distribution of the microspheres to a tibial sample will approach a Poisson distribution, which is approximated

by a normal distribution having a standard deviation equalling the square root of the mean (Godfrey *et al*. 1988). The 'true' number of microspheres that should be in the sample to represent the 'true' blood flow therefore has a 95% probability of lying within the range $n \pm 1.96.\sqrt{n}$, where n is the number of microspheres found in the sample experimentally. For this range to be within 10% either side of n the number of microspheres in each sample must be at least 384 (Katz *et al*. 1971, Buckberg *et al*. 1971). However, if the number of microspheres is only 150, the 95% interval will increase only to $\pm 16\%$ of n, and it has been shown experimentally with tibial samples in the dog that this number of spheres gives comparable calculated flow rates to those obtained with greater numbers (Li *et al*. 1989). The combined results for the different tissues are based on microsphere numbers always in excess of 150 (Table 2.2.3), and the statistical analysis gives similar results to that performed on the individual samples.

The second problem raised by this investigation is that the flows obtained with the Gd^{153} and Sn^{113} labels did not match in the cortical samples. This may have arisen from quenching of the weaker γ-emission of Gd^{153} by the bone mineral, or by problems with the geometry of the samples. Facilities for the decalcification of radioactive bone samples were not available, and so quenching was assessed by another method.

The cortical bone of the rabbit tibia contains approximately 250mg of calcium per gramme dry weight (Paavolainen 1978). All the cortical specimens in the present study had a wet weight of less than 3g, and therefore an overestimate of their calcium content is given by 3×250 or 750mg. There will be much less calcium than this between each microsphere within a cortical sample and the counting crystal. An aliquot of microspheres labelled with Gd^{153} at the base of a counting tube was counted both before and after the addition of 2.08g of calcium chloride, containing 750mg of calcium, dissolved in 10ml of de-ionised water. The calcium was not found to decrease the count rate. This

crude assessment of quenching matches the results of other studies of γ-emitting isotopes (Gross *et al.* 1979, Tøndevold and Eliasen 1982b).

The narrow tubular geometry of the cortical samples differs from that of the muscle and marrow samples and from that of the gelatin columns used for the calibration of the counter (Appendix 1). To overcome this, they were dissolved in acid in order to reproduce the configuration of the microspheres in the reference blood samples, and the method and results of this technique are described below.

2.2.5 CORTICAL SAMPLE REPREPARATION

2.2.5.1 *Method*

Hydrochloric acid was selected for dissolution, as it has a relatively low specific gravity of 1.18 that should not prevent successful centrifugation of the microspheres (specific gravity 1.4). However, in testing aliquots of stock microspheres in acid, it was seen that full-strength acid inhibited the action of the *Decon* detergent, such that the microspheres tended to aggregate to the sides of the tube. Therefore 50% (5.8 N) acid was used for the first six groups of specimens, while full strength acid (11.6 N) was used for the remaining six groups, to be followed by dilution.

Four millilitres of the acid was added to each specimen in its counting tube. No further dissolution appeared to occur beyond 24 hours, but it was noted that the specimens were incompletely dissolved in the half-strength acid, and that samples heavier than approximately 2g were incompletely dissolved in the full-strength acid. Each solution was made up to 10ml by the addition of de-ionised water, and a drop of *Decon* added to each tube. They were centrifuged at 3000 rpm for 15 minutes, and recounted. The heights of the specimens was taken as zero.

To be appended to Section 2.2.5.2

The combined results for the samples dissolved in 11.6 N acid, together with those for marrow and muscle, are shown in Figure 2.2.1.

To assess the effect of the acid on the leaching of label from the microspheres *in vitro*, aliquots of microspheres were placed in the bases of counting tubes and counted. Acid was added at the same time as to the bone samples, and at the time of sample counting these standards were centrifuged and a 1ml sample taken of the supernatants. These supernatant samples were counted, and the calculated activity of the whole supernatant was related to the original activity of the aliquot.

2.2.5.2 *Results*

The results of the recounts of the cortical samples are given in Tables 2.2.5 and 2.2.6. One of the left distal cortical samples of the 5.8 N acid series was lost in the centrifuge. The flows obtained with the two labels remained significantly different after dissolution in the half-strength acid, but after dissolution in full-strength acid only the results for the right proximal cortical samples remained significantly different. The slope of the regression equation for the cortex as a whole was reduced after full-strength dissolution to 1.093 (Table 2.2.7).

The *in vitro* leaching of Gd^{153} was 0.072 ± 0.024 (SE) % in the half-strength acid (n=4), and 0.26 ± 0.13 % in the full-strength acid (n=5). The figures for Sn^{113} were 0.090 ± 0.043 % and 0.79 ± 0.26 % respectively.

2.2.6 DISCUSSION

This further study of the cortical samples has found that dissolution of the bone in 11.6 N acid followed by dilution will give calculated flows for the two labels that are not statistically significantly different when these are injected simultaneously. It appears that the problem posed by the intact samples was one of geometry rather than quenching, and the anomaly of the right proximal cortex is almost certainly due to the incomplete dissolution of some

of the heavier samples, which were always proximal rather
than distal. The corrections applied to allow for specimen
geometry resulted in an overestimation of cortical flow
when the samples were counted intact, with lower calculated
flows found after dissolution.

The ability to accept the null hypothesis of no
difference between two groups of figures depends upon the
sample numbers (Lieber 1990). With the present numbers,
caution is necessary in accepting the hypothesis. While the
cortical flows obtained with the Sn^{113} label tended to be
higher than those obtained with Gd^{153}, the mean differences
between the flows were less than 0.75 ml/min.100g in all
four types of cortical sample. A persistent small
difference between two isotopes has been noted by other
workers (Bell *et al.* 1976), and might be allowed for in
subsequent work either by correcting one or other flow by
the mean difference, or, in experiments measuring flow
sequentially rather than simultaneously, by alternating the
order of use of the isotopes.

Few laboratories appear to have published details of
the accuracy of their isotope separation in biological
samples. Bell *et al.* (1976), using Ce^{141} and Sr^{85} in a
single animal, found flows to tissues of the hindlimb
obtained with the first isotope to approximate 87% of those
with the second, on the basis of linear regression
analysis. Kaihara *et al.* (1968) obtained a figure of
approximately 90% using a similar approach for the isotopes
Sc^{46} and Sr^{85} in four animals. In this study, the
regression coefficients, or slopes, for Gd^{153} and Sn^{113}
were consistently closer to unity than in these studies,
with very high degrees of correlation between the two
isotopes.

As the best correlation between the two isotopes in the
cortex was found in the more completely dissolved samples,
it was noted that in future experiments a slightly larger
volume of full-strength acid would be required for dissol-
ution, and this volume was accordingly increased to 5ml for

the remaining studies. Subjecting the microspheres to strong acid resulted in very low levels of leaching over the period of the bone preparation. Leached isotope will, of course, be included in the specimen count, but leaching will tend to undermine the assumption that all the isotope is at zero height. With the present technique such interference will be negligible.

The microspheres used in this study were smaller than those of 15μm commonly used for bone blood flow estimation. Soft tissue experiments and studies of the pulmonary circulation suggest that microspheres greater than 10μm are not subject to capillary non-entrapment (Section 1.2.4), and no microspheres of the present batches will have been below this size. While facilities for measuring micro-spheres to 0.1μm were not available, microscopical examination of the spheres with a 1μm graticule indicated that they were sized closely around the mean stated by the suppliers. For the purposes of this experiment, the absolute size was not critical, but rather the comparability between the batches labelled with the two different isotopes. These smaller microspheres had the advantage of costing approximately one third the price of 15μm microspheres.

Absolute rates of blood flow in the normal rabbit tibia obtained with the microsphere technique have not previously been reported. Hierton (1983), using 15μm microspheres, quotes rates of 0.5 to 4.0 ml/min.100g in the cortex and 16 to 75 ml/min.100g in the proximal marrow, but his measurements were carried out after at least one week of immobilisation of the opposite hindlimb in full extension, and so cannot be considered to reflect baseline flow. Lunde and Michelson (1970), also with 15μm microspheres, give figures of 1 and 25 ml/min.100g respectively for femoral diaphyseal cortex and marrow. The results of this study are comparable to these figures, and also to flow rates obtained with hydrogen washout in the rabbit and with 15μm microspheres in the cortex of the dog (Table 1.2.1). Marrow

flows in the dog are low, as it is comprised mostly of adipose tissue.

By studying the blood flows obtained *in vivo* with microspheres labelled with the two isotopes, it has been shown that the present technique gives results that allow comparison between opposite limbs, and between flows obtained in the same animal with the two labels. The difficulty has been demonstrated in the obtaining of statistically adequate numbers of microspheres in samples of bone that are both small and the recipients of low levels of blood flow.

Table 2.2.1   Blood flows to the tibial diaphysis and the
muscle of the anterior compartment, measured with 11.3 ±
0.1 (SD) μm microspheres labelled with Gd^{153} and Sn^{113}.
Flows are expressed as mean ± SE ml/min.100g (n=12).

		Gd^{153}		Sn^{113}		P
proximal cortex	R	2.60	± 0.52	3.54	± 0.73	<0.002
	L	2.64	0.58	3.38	0.72	<0.01
distal cortex	R	3.21	0.89	4.23	1.15	<0.01
	L	3.31	0.86	4.45	1.21	<0.02
proximal marrow	R	40.17	6.14	42.70	6.27	NS
	L	37.86	7.15	40.42	7.23	NS
distal marrow	R	13.73	2.85	12.58	2.73	NS
	L	12.66	2.05	12.62	2.24	NS
skeletal muscle	R	12.24	2.13	13.22	2.53	NS
	L	11.56	1.78	11.50	1.83	NS

R = right L = left NS = not significant at 5% level (paired t test)

Differences between limbs all NS

Table 2.2.2 The number of microspheres labelled with Gd^{153} and Sn^{113} counted in samples of the tibial diaphysis and skeletal muscle (mean to nearest integer $\pm$ SE, n=12).

		Gd^{153}		Sn^{113}	
proximal cortex	R	209	$\pm$ 38	263	$\pm$ 48
	L	211	33	263	48
distal cortex	R	217	49	261	53
	L	226	46	269	49
proximal marrow	R	827	132	865	134
	L	870	159	893	151
distal marrow	R	207	35	190	31
	L	200	27	194	28
skeletal muscle	R	894	239	928	234
	L	803	213	836	236

R = right   L = left

Table 2.2.3 Blood flows to the tissues of the tibial diaphysis and the muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres labelled with Gd[153] and Sn[113]. Flows are expressed as mean ± SE ml/min.100g (n=12).

	Gd[153]		Sn[113]		p
cortex	2.90 ± 0.68		3.86 ± 0.90		<0.01
marrow	27.70	4.42	28.85	4.52	NS
muscle	11.93	1.90	12.46	2.03	NS

NS = not significant at 5% level (paired *t* test)

Table 2.2.4 Linear regression analysis of the relationship between the flows obtained with microspheres labelled with Gd^{153} and Sn^{113} (n=12). The dimension of flow is ml/min.100g.

$$Sn^{113} \text{ flow} = \text{intercept} + (\text{slope} \times Gd^{153} \text{ flow})$$

		Intercept	Slope	r	p
proximal cortex	R	-0.020	1.369	0.985	<0.001
	L	0.254	1.186	0.956	<0.001
distal cortex	R	0.191	1.260	0.981	<0.001
	L	-0.161	1.394	0.989	<0.001
proximal marrow	R	2.889	0.991	0.972	<0.001
	L	2.814	0.993	0.982	<0.001
distal marrow	R	0.364	0.889	0.929	<0.001
	L	-0.633	1.047	0.960	<0.001
skeletal muscle	R	0.007	1.080	0.911	<0.001
	L	0.082	0.988	0.961	<0.001
whole cortex		0.061	1.307	0.987	<0.001
whole marrow		0.978	1.006	0.984	<0.001
whole muscle		0.256	1.022	0.959	<0.001

R = right   L = left r = linear correlation coefficient

Table 2.2.5 Blood flows to the cortex of the tibial diaphysis measured with 11.3 ± 0.1 (SD) µm microspheres labelled with Gd^{153} and Sn^{113}, counted after dissolution in 5.8 N hydrochloric acid. Flows are expressed as mean ± SE ml/min.100g (n=6).

		Gd^{153}		Sn^{113}		p
proximal cortex	R	1.95	± 0.54	2.44	± 0.61	<0.02
	L	1.86	0.41	2.45	0.60	<0.05
distal cortex	R	2.42	0.80	2.77	0.69	NS
	L†	2.43	0.74	3.01	0.81	NS

R = right   L = left   NS = not significant at 5% level (paired *t* test)

† n=5 (one specimen lost in centrifuge)

Table 2.2.6 Blood flows to the cortex of the tibial diaphysis measured with 11.3 ± 0.1 (SD) μm microspheres labelled with Gd[153] and Sn[113], counted after dissolution in 11.6 N hydrochloric acid. Flows are expressed as mean ± SE ml/min.100g (n=6).

		Gd[153]	Sn[113]	p
proximal cortex	R	1.75 ± 0.50	2.15 ± 0.55	<0.05
	L	1.87 0.67	2.03 0.59	NS
distal cortex	R	2.75 1.51	3.25 1.58	NS
	L	2.72 1.25	3.45 1.61	NS

R = right   L = left   NS = not significant at 5% level (paired *t* test)

Table 2.2.7 Linear regression analysis of the relationship of cortical flows obtained with microspheres labelled with Gd^{153} and Sn^{113} after dissolution in 11.6 N hydrochloric acid (n=6). The dimension of flow is ml/min.100g.

$$Sn^{113} \text{ flow} = \text{intercept} + (\text{slope} \times Gd^{153} \text{ flow})$$

		Intercept	Slope	r	p
proximal cortex	R	0.252	1.083	0.975	<0.001
	L	0.380	0.883	0.997	<0.001
distal cortex	R	0.394	1.038	0.992	<0.001
	L	-0.038	1.284	0.998	<0.001
whole cortex		0.230	1.093	0.995	<0.001

R = right   L = left r = linear correlation coefficient

Figure 2.2.1 Blood flows to the tissues of the tibial diaphysis and the muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres labelled with Gd153 and Sn113. The figures for cortical flow are those obtained after dissolution in 11.6 N hydrochloric acid.
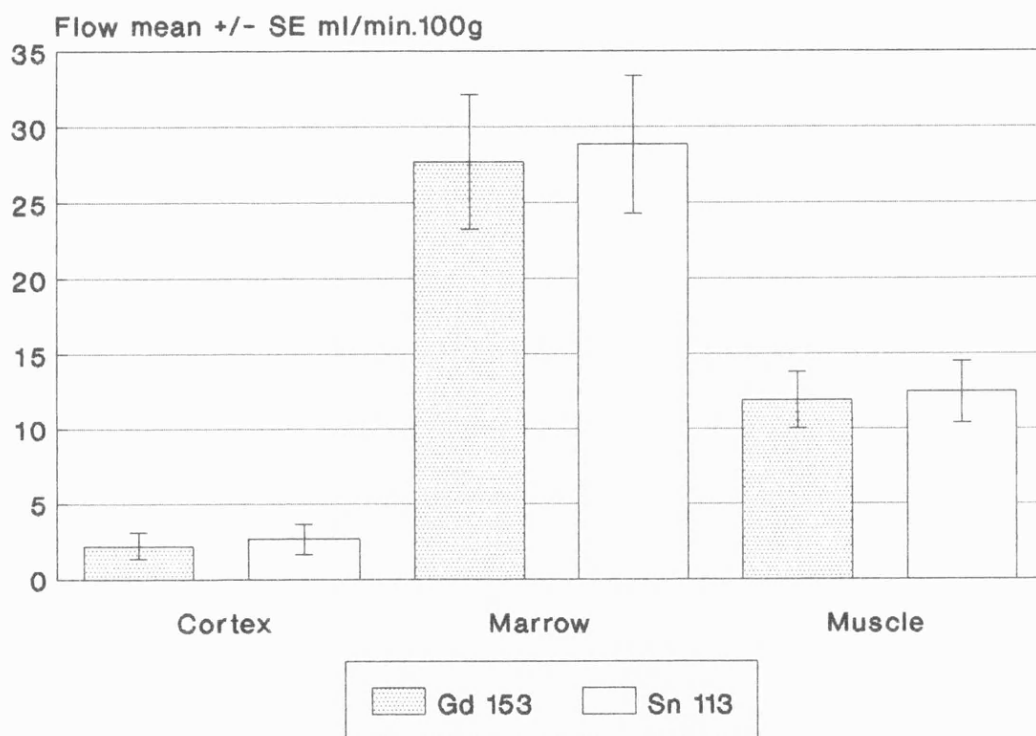
2.3

A COMPARATIVE STUDY OF BLOOD FLOWS TO THE TIBIAL DIAPHYSIS OF THE ADULT RABBIT OBTAINED WITH 10μm AND 15μm RADIOACTIVE MICROSPHERES

2.3.1 INTRODUCTION

Almost all microsphere studies of bone blood flow have been undertaken using 15μm spheres. This size was originally selected in preference to the then popular 50μm by Lunde and Michelsen (1970), on the basis of Brånemark's figure for Haversian canal diameter in the rabbit tibia (Brånemark 1959).

In this animal, 50μm microspheres have been found to be subject to streaming in the aortic arch (Warren and Ledingham 1974) and to axial streaming in a branch of the femoral artery (Phibbs and Dong 1970). They are more likely to cause central nervous system side-effects than 15μm microspheres (Warren and Ledingham 1974), and they are considerably more expensive. A more recent study comparing 50μm to 15μm spheres showed that the distribution between cortex and marrow was shifted in favour of the cortex with 15μm spheres, suggesting hindrance of the passage of the larger spheres in the nutrient circulation (Gregg and Walder 1980).

Little information is available as to the calibre of intra-osseous vessels. The problem is compounded by the uncertainty as to which vessels any circulating microspheres will first encounter. De Bruyn et al. (1970), in an ink perfusion study of the long bones of guinea pigs, rats and rabbits, described vessels passing between the cortex and marrow with a calibre of only 2 to 4μm, similar to the figure of 5μm given by Brookes (1987). Within the

cortex, Brookes (1971) refers to the vessels of the Haversian canals as 15µm to 30µm in diameter, while de Bruyn's group describe longitudinal vessels of 10 to 14µm running obliquely, emptying into both periosteal vessels and the peripheral medullary sinusoids. With vessels as small as this, a difference of 1 or 2µm in microsphere diameter may possibly have a profound influence on their intravascular distribution.

The kidney cortex provides a good model for the study of steric hindrance of microsphere distribution, having a well-known vascular structure which includes a predominantly unidirectional system of supply vessels (Mørkrid *et al.* 1976). Using spheres of different sizes, Katz *et al.* (1971) confirmed that the 'trapping elements', *ie*, the vascular locations of microsphere arrest, varied with microsphere size. Thus, the measured blood flow varied with the distribution of the trapping elements. For example, virtually all 15µm microspheres were trapped in glomeruli, and therefore the apparent flow to the subcapsular layer of cortex was very low, there being very few glomeruli in this location. Secondly, microspheres larger than 15µm were preferentially trapped in the outer cortex, suggesting that they were too large to leave the interlobular arteries more proximally. Microspheres of 35µm were 'almost all' trapped in the interlobular arteries or afferent arterioles rather than in the glomeruli. Microscopic analysis of trapped microspheres of mixed sizes has shown dramatic changes of distribution within the cortex with a change in size of just 1µm, from 21µm to 22µm (Mørkrid *et al.* 1976).

These findings emphasise the importance of steric factors in the distribution of microspheres within an organ. However, this factor has received scant attention in the study of bone blood flow, and it has been generally believed that the size closest to that of erythrocytes while large enough to prevent non-entrapment is 15µm. Pooled results in the dog for sternum, rib, femoral inferior epiphysis, diaphyseal cortex and marrow showed that 9 ± 1 (SD) µm spheres gave flow rates 34% less than

those given by 15μm and 25μm spheres, suggesting considerable non-entrapment (Gross et al. 1979), but such pooling of results obscures differences between tissues, particularly between cortex and marrow. In a study of the cancellous bone of the canine femoral head, Niv and Hungerford 1979) found that 9 ± 1 μm microspheres gave results "up to fifty per cent" lower than those given by 15μm microspheres. However, this difference may represent the lower half of the size distribution of the smaller spheres, as microspheres less than 9μm in diameter have been shown to be responsible for the great majority of systemic non-entrapment (Section 1.2.4).

In order to follow most closely the distribution of erythrocytes within arterial lumens (Phibbs and Dong 1970) and within each organ, the microspheres should be as close in size as possible to these cells while remaining large enough to avoid non-entrapment in the capillary bed. From soft tissue studies, this ideal size would appear to be approximately 10μm (Section 1.2.4). Wootton and Doré (1986) have used microspheres of 11.1 ± 0.3 μm in an assessment of fluorine-18 extraction by bone in the rabbit. After distal aortic injection, they found the venous to arterial ratio of microspheres in the femoral nutrient vessels to be only 0.24%, while overall non-entrapment in the lower body as estimated by lung counting was only 1.1%. It may be pointed out that the nutrient veins of long bones constitute only a fraction of the total venous drainage (Oni, Stafford and Gregg 1987,1988), and the difference from the results of the canine studies may be due to species differences. However, the responsible factor giving rise to the low lung counts was probably the 2μm difference between the sizes of the microspheres used in the two different species.

It was therefore decided that a preliminary experiment should be carried out in the rabbit, directly to compare flows obtained in the tibial shaft with microspheres nominally sized 10μm and 15μm.

2.3.2 METHOD

The study was performed on six adult female New Zealand
White rabbits weighing 3.2 to 4.35kg. The method employed
was that described in Section 2.2, except that the
injectate comprised a mixture of approximately 2×10^6
microspheres sized 16.5 ± 0.1 (SD) μm and approximately
4×10^6 microspheres sized 11.3 ± 0.1 μm labelled with
Gd[153] and Sn[113]. The number of the larger microspheres was
reduced after it had been noticed previously that inclusion
of a larger number in a combined injectate occasionally
caused systemic arterial hypotension. The labels for each
size were alternated, so that half the animals received the
larger microspheres labelled with Gd[153] and the smaller
microspheres labelled with Sn[113], while the other half
received the opposite. The cortical bone samples were
prepared by dissolution in 5mls of 11.6N hydrochloric acid
(Section 2.2.5).

The differences between the flows obtained with the two
sizes of microsphere were subjected to the paired *t* test.

2.3.3 RESULTS

2.3.3.1 *Animal preparation*

All microsphere injections and withdrawal samples were
completed within 62 minutes of induction of anaesthesia. In
two animals the injection of the microspheres caused a drop
in mean arterial pressure of 5mmHg.

2.3.3.2 *Comparative blood flows*

The mean flows obtained are given in Table 2.3.1. The full
results are given in Table A.3.4, Appendix 3.

In the cortex and distal marrow, calculated flows with
the smaller microspheres were greater than with the larger,
but the opposite was found in the proximal marrow and

skeletal muscle. However, none of these differences were found to be statistically significant at the 5% level.

While many of the cortical and distal marrow samples contained less than 150 of the larger microspheres (Section 2.2.4), the combined results for the three types of tissue represent numbers of microspheres greater than this, except for the muscle count of the larger microspheres in one animal and the cortex counts of the larger microspheres in this and another animal. This will raise the variability of the calculated flow rate slightly. The combined flows were not found to be significantly different (Table 2.3.2, Figure 2.3.1).

The results obtained for blood flows with the 11.3μm microspheres are similar to those obtained in the previous study (Tables 2.2.1 and 2.2.6). Taking the average of the flows obtained with the two isotopes in that study and comparing with the present results using the unpaired *t* test gives *p* values ranging from >0.2 to >0.9 for the different tissue samples.

2.3.4 DISCUSSION

In any tissue, microspheres smaller than 15μm and closer to the size of the erythrocytes might be expected to reflect whole blood flow more accurately, and hindrance of the entry of microspheres into a portion of tissue as a result of their size being in excess of that of the supplying vessels has been termed *steric restriction* (Mørkrid *et al*. 1976). Any reduction in this restriction obtained by reducing microsphere size might be offset by a concurrent increase in non-entrapment, resulting from passage of the microspheres through small arteriovenous shunts and through the capillary bed.

In view of the multiplicity of the arterial and venous vessels comprising the blood supply and drainage of a long bone, it is not possible at present to perform direct measurements of non-entrapment in bone. Methods involving

injection of microspheres directly into the nutrient artery
(Morris and Kelly 1980, Tothill, Hooper, Hughes and
McCarthy 1987) give results applicable to only one of these
channels, and also involve surgical interference. Up to the
present this technique has been applied only to 15μm
microspheres. Other methods involving the sampling of mixed
venous blood or of the lungs result in the inclusion of
microspheres passing through non-osseous tissues,
particularly those shunting through cutaneous arteriovenous
anastomoses (Spence *et al*. 1972, Zanick and Delaney 1973).
In view of these difficulties, the degree of non-entrapment
has previously been concluded from differences in the
calculated flows obtained with microspheres of different
sizes (Gross *et al*. 1979, Niv and Hungerford 1979).

Using this indirect assessment, any differences in the
restriction of the two sizes of microsphere occurring at
the cortex-marrow interface would not be expected to affect
the flows obtained for the diaphysis as a whole, and
combining mathematically the cortical and marrow flows, the
average flows for the two sizes are virtually identical at
6.43 ± 0.48 (SE) ml/min.100g for the 11μm and 6.37 ±
0.35 ml/min.100g for the 16μm microspheres ($p > 0.8$). Thus
the comparative flows obtained in diaphyseal bone and
skeletal muscle suggest that microspheres tightly sized
around a mean of approximately 11μm are not more subject to
non-entrapment than 16μm microspheres, and this is in
agreement with the results of venous sampling of
microspheres across non-osseous tissues in the lamb and dog
(Katz *et al*. 1971, Archie *et al*. 1973, Gaehtgens *et al*.
1976). While the smaller microspheres tended to give
slightly higher cortical flows consistent with a reduction
in steric restriction, this effect was not statistically
significant.

This study has shown that 11μm microspheres are
suitable for the measurement of blood flow to the tibial
diaphysis. Apart from the potential rheological and steric
advantages of these microspheres, the very low level of
blood flow to the tibial cortex requires injectates to

contain large numbers of microspheres, and the greatly
reduced cost of microspheres nominally sized 10μm results
in considerable economic savings.

Table 2.3.1   Blood flows to the tibial diaphysis and the muscle of the anterior compartment, measured with 16.5 ± 0.1 (SD) μm and 11.3 ± 0.1 μm microspheres. Flows are expressed as mean ± SE ml/min.100g (n=6).

		16μm		11μm		p
proximal cortex	R	1.79	± 0.67	2.31	± 0.93	NS
	L	1.67	0.53	2.28	0.79	NS
distal cortex	R	2.43	0.80	2.77	1.03	NS
	L	2.49	0.70	2.77	0.75	NS
proximal marrow	R	37.89	4.10	34.11	5.48	NS
	L	37.16	5.12	33.19	3.86	NS
distal marrow	R	13.12	2.43	13.83	2.15	NS
	L	12.41	1.47	14.29	3.05	NS
skeletal muscle	R	13.77	2.73	13.35	2.43	NS
	L	18.34	6.15	16.62	5.52	NS

R = right   L = left   NS = not significant at 5% level (paired t test)

Table 2.3.2   Blood flows to the tissues of the tibial diaphysis and the muscle of the anterior compartment, measured with 16.5 ± 0.1 (SD) μm and 11.3 ± 0.1 μm microspheres. Flows are expressed as mean ± SE ml/min.100g (n=6).

	16μm	11μm	p
cortex	2.07 ± 0.66	2.51 ± 0.87	NS
marrow	26.53 3.33	24.92 3.27	NS
muscle	15.57 4.07	14.54 3.65	NS

NS = not significant at 5% level (paired *t* test)

Figure 2.3.1   Blood flows to the tissues of the tibial diaphysis and the muscle of the anterior compartment, measured with 16.5 ± 0.1 (SD) μm and 11.3 ± 0.1 μm microspheres (n=6).
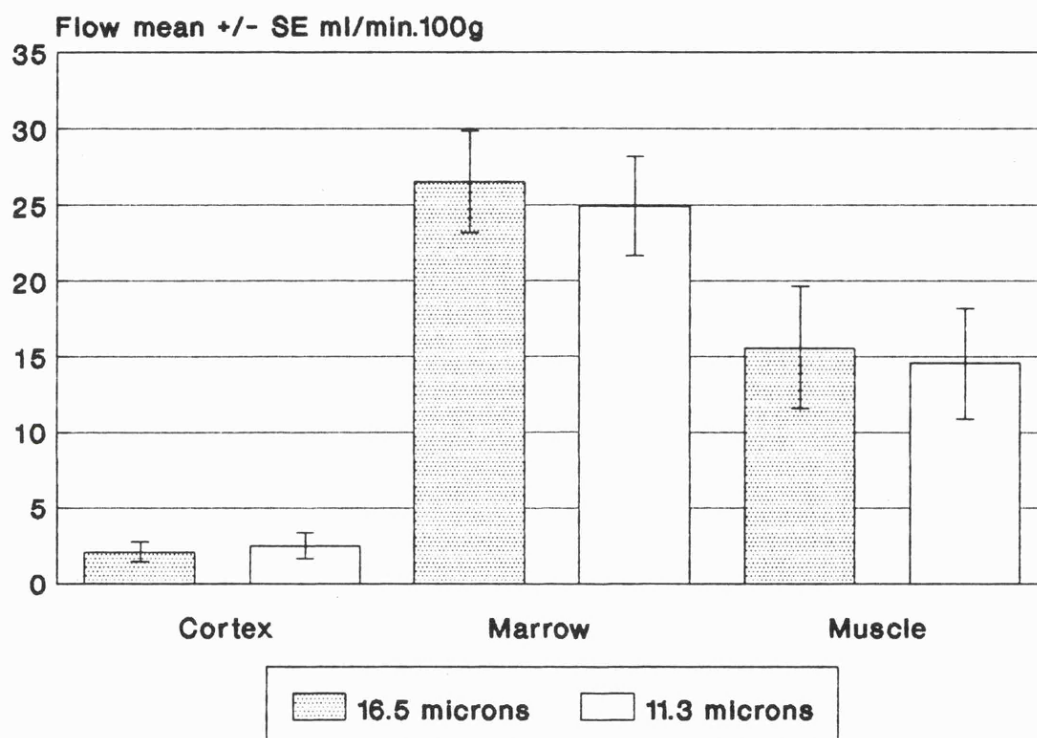
2.4

SEQUENTIAL MEASUREMENTS OF BLOOD FLOW TO THE TIBIAL DIAPHYSIS OF THE ADULT RABBIT USING RADIOACTIVE MICRO-SPHERES

2.4.1 INTRODUCTION

The facility for labelling microspheres with different radio-isotopes enables measurement of blood flow on more than one occasion in each animal. While the number of microspheres injected should be large in order to reduce random variation in their distribution, large numbers may potentially cause central side effects, and might reduce organ blood flow by excessive embolisation of the microvasculature (Section 1.2.4). Subsequent measurements would then be affected.

In the rat, Schoutens et al. (1979) tested the effect of increasing the number of injected 15μm microspheres on the calculated bone blood flow obtained with a second injection in the same animal. In this species, the maximum size of the injectate that did not reduce tibial and femoral flow was of the order of 3.5×10^5 microspheres, or approximately 1.4×10^6 microspheres per kilogram body weight. In the dog, femoral flows are not significantly affected by injections of 2×10^6 15μm microspheres (Gross et al. 1979, Jones et al. 1982). No similar studies have been reported using 11μm microspheres or in the rabbit, although Hierton (1981), investigating the effect of various drugs on blood flow, noted that flow to the femoral diaphysis was unchanged by a previous injection of 1.7×10^6 15μm microspheres after an intermediate infusion of either naproxen or paracetamol. Hales and Cliff (1977) observed microspheres in vivo in rabbit ear chambers and

noted that lodgement of microspheres resulted in rapid opening of collateral flow, and often did not prevent the passage of erythrocytes along the same vessel. This suggests that the number of injected microspheres required to cause local haemodynamic upset in the rabbit would be very large.

The experiment in Section 2.2 has shown that up to 12 × 10^6 11μm microspheres may be injected in the anaesthetised rabbit without any apparent systemic side-effects. The maximum dosage of microspheres in subsequent studies was limited by the radioactive disposal limits (Section 2.1.3.4), but the experiment described in this section was performed to ensure that the doses of microspheres to be used in later studies was insufficient to cause a reduction in the blood flow of the tibial diaphysis.

2.4.2 METHOD

The study was performed on six female New Zealand White rabbits weighing 3.25 to 4.50kg. Tibial diaphyseal and skeletal muscle blood flow was measured as previously described (Section 2.2), using injectates of 3.1-5.8 × 10^6 microspheres sized 11.3 ± 0.1 μm.

Two measurements of blood flow were made in each animal. The label of the first injectate was alternated between Gd^{153} and Sn^{113}. The volume of blood taken into the first withdrawal sample was replaced by the saline flush used during the first injection (approximately 5mls) followed by the intravenous injection of 5mls of *Haemaccel* (Hoechst UK Ltd, Hounslow), a polygeline plasma substitute. The second measurement was made six to eight minutes after the first, using the other label.

The differences between the first and second measurements for each tissue were subjected to the paired *t* test. The effect of increases in the numbers of microspheres administered in the first injection on the flows obtained with the second was analysed by linear

regression, expressing the ratio of the two measurements to the number of microspheres per kilogram body weight administered in the first injection.

2.4.3 RESULTS

2.4.3.1 *Animal preparation*

There were no consistent changes in blood pressure and heart rate between the two measurements, and no episodes of hypotension were observed after any of the injections.

2.4.3.2 *Blood flows*

The mean flows obtained are given in Table 2.4.1, and the combined means for the three tissues in Table 2.4.2 and Figure 2.4.1. The results in full are presented in Table A.3.5, Appendix 3.

On average, flows for the bony tissues were higher on the second measurement, while those for skeletal muscle were lower, but these differences were not statistically significant. On linear regression analysis, no relationship was found between the ratio of the second measurement to the first measurement and the number of microspheres per kilogram body weight administered in the first injection, with all correlation coefficients found to be less than 0.3 and all slopes insignificantly different from zero.

The results obtained for blood flows with the first injection of microspheres are similar to those obtained in the study of simultaneous injections (Tables 2.2.1 and 2.2.6). On taking the average of the flows obtained with the two isotopes in that study and comparing with the present results using the unpaired *t* test, no significant differences were found for any of the different tissue samples, the *p* values ranging from >0.3 to >0.9.

Many of the individual samples contained less than 150 microspheres, particularly after the lower dosages, but of

the combined tissue samples only one of the measurements
contained less than this number.

2.4.4 DISCUSSION

It is probable that the proportion of cardiac output
delivered to the tibial diaphysis varies little with the
body weight of the animals. However, larger animals will
have a greater total number of intra-osseous vessels, and
thus larger numbers of microspheres will be required to
cause haemodynamically significant embolisation. The local
effects of the microspheres would therefore be expected to
vary with the injected number per unit body weight. In this
study, this number varied two-fold, but the ratios of the
second to the first flow measurements showed no tendency to
vary with this number. This study has therefore shown that
an injection of 11μm microspheres with a range of dosage of
approximately 0.8 to 1.8 × 10^6 per kilogram body weight
does not adversely affect tibial diaphyseal blood flow.
Indeed, in most animals the osseous flow increased
slightly, possibly as a result of the opening of
collaterals observed by Hales and Cliff (1977).

The technique developed is thus suitable for the
measurement of tibial and skeletal muscle blood flow on two
occasions in the same animal, enabling paired analysis of
experimental interventions.

Table 2.4.1   Blood flows to the tibial diaphysis and the muscle of the anterior compartment, measured sequentially with 11.3 ± 0.1 µm (SD) microspheres. Flows are expressed as mean ± SE ml/min.100g (n=6).

		First		Second		p
proximal cortex	R	2.36 ±	0.50	2.68 ±	0.56	NS
	L	2.01	0.53	2.35	0.62	NS
distal cortex	R	2.49	0.61	2.87	0.85	NS
	L	2.43	0.69	2.96	0.79	NS
proximal marrow	R	42.96	10.47	56.27	14.19	NS
	L	42.26	10.66	52.33	12.80	NS
distal marrow	R	17.53	3.89	23.28	5.78	NS
	L	13.93	3.43	20.35	5.23	NS
skeletal muscle	R	9.81	2.84	6.59	1.29	NS
	L	9.54	3.45	6.43	1.47	NS

First = first measurement   Second = second measurement

R = right   L = left   NS = not significant at 5% level (paired *t* test)

Table 2.4.2 Blood flows to the tissues of the tibial diaphysis and the muscle of the anterior compartment, measured sequentially with 11.3 ± 0.1 μm (SD) microspheres. Flows are expressed as mean ± SE ml/min.100g (n=6).

	First	Second	p
cortex	2.33 ± 0.53	2.70 ± 0.65	NS
marrow	30.57 6.79	39.24 8.96	NS
muscle	9.70 3.11	6.48 1.36	NS

First = first measurement   Second = second measurement

NS = not significant at 5% level (paired *t* test)

Figure 2.4.1 Blood flows to the tissues of the tibial diaphysis and the muscle of the anterior compartment, measured sequentially with 11.3 ± 0.1 (SD) µm microspheres (n=6).
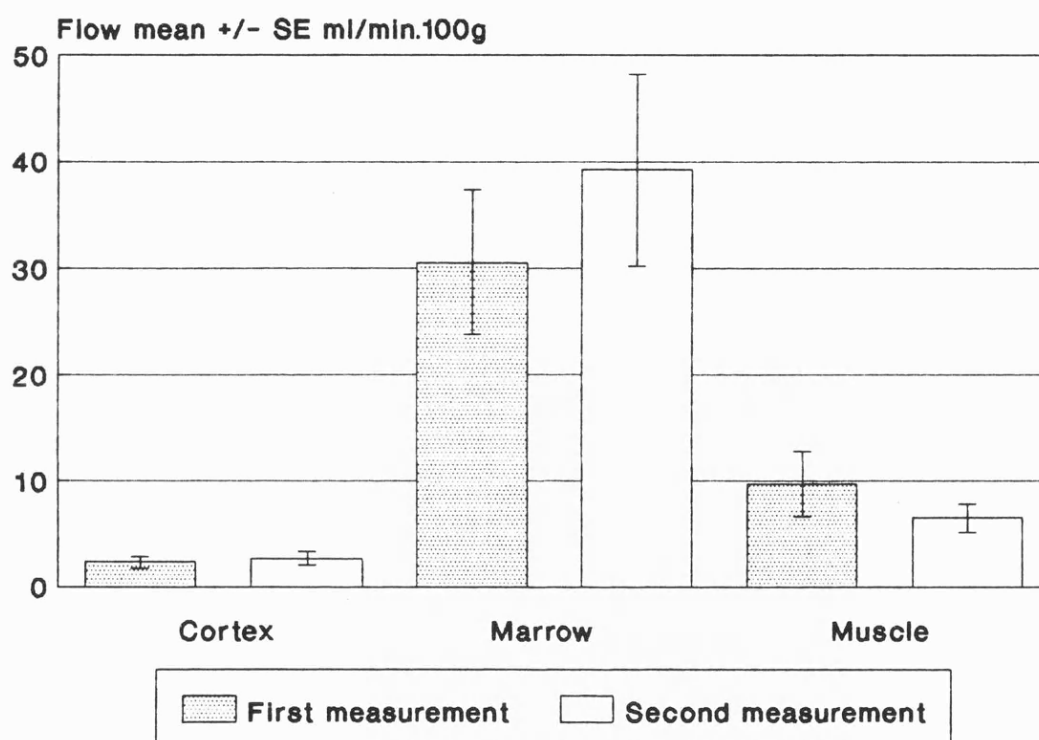
2.5 —————————————————————————————

A STUDY OF THE EFFECT OF CAST IMMOBILISATION ON BLOOD FLOW TO THE TIBIAL DIAPHYSIS OF THE ADULT RABBIT

2.5.1 INTRODUCTION

It is clear that different methods of fixation are associated with differences in bone blood flow changes during fracture healing (Section 1.3.2). In order to eliminate the effects of the insertion of pins or plates into or onto the tibia, the hindlimb may be immobilised in a cast, but the effects of this form of management should be known so that they are not confused with the results of the intervention under study. Further, it is desirable to use the contralateral unfractured limb as a control, in order to reduce the statistical problem posed by the wide variation between different animals, but the use of this limb is likely to change during the experimental period and it cannot be assumed that the 'control' limb is unaffected by the immobilisation of the experimental limb. There is, however, very little quantitative information as to the effects of simple immobilisation on blood flow to the bones of the hindlimbs.

Several studies in the rabbit using angiography after plaster immobilisation of one hindlimb have been reported. Geiser and Trueta (1958) noted an increase in the number of vessels visualised around the os calcis, and that this change commenced in the first week. Interestingly, they did not find any differences between these animals and those also subjected to an oblique osteotomy of the tibia held by circlage wiring. Ferguson and Akahoshi (1960) described vessel proliferation around the knee, which was included in the cast in their series. No changes were noted in the

number of vessels of the muscles below the knee. Hulth and
Olerud (1960a,b) found that the major vessels of the
hindlimb became more tortuous during the second week, and
that in some cases this change was bilateral.

An attempt to quantify this apparent increase in
vascularity was made by Semb (1968,1969), who compared the
uptake of Sr^{85} in the two limbs of the rabbit. After one or
three weeks of immobilisation, the experimental tibia had a
higher uptake of the isotope, but this difference was not
statistically significant. Hierton (1983) studied a model
of myositis ossificans in the rabbit, in which one hindlimb
was splinted in extension and flexed daily out of the
splint after the first week. After one week of this regime,
two weeks after application of the splint, there were
reductions in the flow to the proximal tibia with respect
to the control limb as measured by the microsphere
technique. After a further week, there were large
reductions in flow to the tibial marrow and smaller
reductions in flow to the cortex and anterior compartment
muscle. This result may have arisen from the unphysio-
logical position of the limb with consequent heavy usage of
the 'control', as the differences between the one week and
two week groups appears to have arisen from increases in
the flow to the control limb rather than falls in the
splinted limb. Further, the technique used in this study,
involving the taking of withdrawal samples from the *cranial*
end of a carotid artery, is of questionable validity, and
the tables of results suggest that some values are quoted
incorrectly by a factor of one hundred.

In view of the lack of quantitative data on this
subject, it was necessary before cast immobilisation was
used in the study of experimental fractures to investigate
the result of casting alone on blood flow to both the
immobilised and the control limb. This could be carried out
using the sequential method of measurement of bone blood
flow studied in the previous section (Section 2.4). Using

non-biodegradable microspheres, these measurements may be
performed at times far removed from one another.

Hales and Cliff (1977) observed the fate of injected
microspheres sized 13.7 ± 4.1 μm *in vivo* using rabbit ear
chambers. The majority of spheres moved within the chamber
over the first two days, and approximately 20% moved
sufficiently far to leave a high power microscope field.
However, counts over the ear and the lungs remained
constant over the course of fifty days, suggesting that
return of the microspheres to the systemic circulation was
negligible. These authors also observed that the
microspheres did not excite any cellular response, and that
the effects on the local circulation appeared minimal.
Thus, blockage of a vessel by a microsphere resulted in
redirection of flow through collaterals within thirty
seconds, and many spheres did not prevent the passage of
erythrocytes along the vessel in which they had lodged. By
three weeks, almost all the microspheres were extra-
vascular.

The fate of extravascular microspheres has been
investigated in the dog (Robertson *et al.* 1985). After
direct injection into the femur, many 15μm microspheres
passed directly into the venous system, but the remainder
showed no tendency to move to the lungs or into the
lymphatic system over the course of four days. A small
number of microspheres were found in local lymph nodes
after injection into skeletal muscle alongside the femur,
but 92% of spheres had remained at the injection site at
four days. Thus, microspheres injected intravascularly may
be cleared in small numbers from muscle once they have
become extravascular, but this does not appear to happen in
the short-term in diaphyseal bone.

Hales and Cliff (1977) observed that there was no
leaching of isotope into the urine or faeces from
microspheres labelled with Ce[141], although this was found
to occur with spheres labelled with I[125]. DuPont were
unable to furnish any data concerning leaching from their

Nen-Trac microspheres *in vivo*, and therefore this was tested in a pilot experiment.

In order to obtain two measurements of blood flow in the same animal on different days, the catheters may be left *in situ* or they may be removed and subsequently reinserted into the same vessels. Both options present technical difficulties, and the good mixing of the microspheres obtained with the present technique suggested that the second measurement could be obtained by catheterising the contralateral carotid and brachial arteries. The effects of occluding both carotid arteries is minimal in the rabbit (Section 2.1.2.2), but two pilot experiments were carried out to ensure that left and right brachial sampling were equivalent.

2.5.2 METHOD

2.5.2.1 *Isotope leaching*

The pilot experiment was carried out on a female New Zealand White rabbit weighing 3.85kg. The animal was prepared as previously described (Section 2.2.2), except that the catheterisations were performed aseptically. A mixture containing 8.0×10^6 microspheres sized 11.3 ± 0.1 μm, with one half labelled with Gd^{153} and the other with Sn^{113}, was injected via the aortic catheter, and the animal allowed to recover following removal of the catheters. It was kept in a metabolic cage for a period of two weeks, with collection of all urine and faeces passed.

The collections from each day were weighed and two samples taken from each. These were weighed and counted for leached label. After correction for decay, the amount of label in the whole collection for each day was calculated, and expressed as a percentage of the administered dose.

2.5.2.2 *Brachial sampling*

Two female New Zealand White rabbits weighing 3.95 and 4.05kg were studied. After preparation as before, catheters were inserted into the ascending aorta via both carotid arteries, and both brachial arteries were cannulated. Injections containing 1 × 10⁶ microspheres sized 11.3 ± 0.1 μm and labelled with Sn¹¹³ were given alternately via each carotid cannula, while withdrawal samples were taken simultaneously from both brachial arteries. The volume of blood withdrawn was replaced with *Haemaccel* before the next injection. Five injections were given in the first animal, and three injections in the second before one of the brachial catheters became occluded.

The withdrawal samples were counted after centrifugation. As the two withdrawal syringes gave slightly different withdrawal rates, the counts were corrected to unit withdrawal rate before comparison.

2.5.2.3 *Cast immobilisation*

The effect of cast immobilisation was investigated in twelve female New Zealand White rabbits weighing 4.05 to 5.35kg. Measurements of blood flow were made with injections of 3.0-4.8 × 10⁶ microspheres sized 11.3 ± 0.1 μm, with alternation of the label used for the first injection. The upper limit of the number of microspheres in any one animal was imposed by the isotope disposal limits after the discontinuation of the lower activity microspheres by the manufacturer (Section 2.1.3.4). The first measurement was performed aseptically as above, but before recovery from the anaesthetic one hindlimb was selected at random by the toss of a coin, and immobilised in a padded water-resistant polyurethane cast (*Delta-Cast*, Johnson and Johnson, New Milton) from the toes to just above the knee, with the foot at right-angles or slightly dorsiflexed to allow the animal to sit comfortably. For the

first day postoperatively the animals were caged, but thereafter were kept in the free-range room.

The second blood flow measurement was performed after one week in six animals and after two weeks in the other six. The animals were again anaesthetised, and a repeat blood flow measurement performed using microspheres of the opposite label. For this measurement, the catheters were inserted via the right carotid artery and the right brachial artery. The bone and skeletal muscle samples were prepared and counted as previously described (Sections 2.2.2 and 2.2.5).

The flows before and after immobilisation in each group were compared using the paired t test, while the flows after one and two weeks of immobilisation were compared using the unpaired t test.

2.5.3 RESULTS

2.5.3.1 *Isotope leaching*

The animal did not show any ill-effects from the injection, nor from the ligation of the left carotid and brachial arteries.

The results of the leaching counts are given in Table 2.5.1. It is clear that the degree of leaching was minimal, amounting to less than 0.5% for each isotope over the whole experimental period, and thus leaching of the isotopes is unlikely to affect significantly the results of flow measurements performed up to two weeks before sacrifice of the animals.

2.5.3.2 *Brachial sampling*

At post mortem the side ports of the carotid catheters were found in both animals to be side-by-side just above the aortic valve, and therefore the results of the left and right injections were considered together. The left and

right brachial samples were not found to differ
significantly on paired *t* testing, although strictly this
test is not applicable as the eight injections were not
completely independent. The counts were higher on the right
side on four occasions and on the left on four, and the
geometric means of the ratios of right to left were 1.20
for the first animal and 1.01 for the second.

2.5.3.3 *Cast immobilisation*

The animals did not appear unduly restricted by the casts,
and moved about the room normally. Most lost a little
weight over the first two to four days postoperatively, and
several were noted to gnaw at either end of the cast. The
mean flows in the two groups both before and after
immobilisation are given in Tables 2.5.2 and 2.5.3, and
graphically in Figures 2.5.1 and 2.5.2. The full results
are given in Tables A.3.6 and A.3.7, Appendix 3.

The flows to the cortex and marrow decreased after one
week of immobilisation in both limbs, while the flow to the
muscle increased, but these changes were not statistically
significant. In the two week group of animals the cortical
flows remained slightly decreased, while the marrow flows
had now increased, together with the flow to the muscle.
Again these changes were not statistically significant.
Flows before casting were not significantly different
between the one week and two week groups on unpaired *t*
testing, while after casting those to the proximal marrow
were significantly higher in the two week group in both the
immobilised and control limbs ($p < 0.05$).

All of these changes occurred in both immobilised and
control limbs, and in both the one week and the two week
groups the flows to the immobilised limb did not differ
significantly from those to the control either before or
after casting.

As a result of the relatively small number of
microspheres that could be used in this experiment, most

tissue samples contained less than 150 microspheres.
However, the variability of blood flows between animals was
not higher than in the previous studies, except in the
distal marrow where the coefficients of variation were
twice that of the previous studies. This arose from the
lower flows in these predominantly older animals, which in
combination with the small size of these specimens (all
less than 0.7g) resulted in their containing low numbers of
microspheres.

2.5.4 DISCUSSION

The pilot studies demonstrated that the sequential method
of measurement of blood flow could be performed accurately
over a two week period using different catheter sites. The
results of the brachial sampling experiment confirm the
adequacy of mixing suggested by the equality of left and
right tibial flows of earlier experiments (Section 2.2) and
by the results of other studies using aortic injection
(Lunde and Michelson 1970, Gregg and Walder 1980, Aalto and
Slätis 1984, Court-Brown 1985, Wootton and Doré 1986,
Section 2.1.2.2). The use of larger numbers of microspheres,
as in the immobilisation experiment, is likely to reduce
proportional random variation further.

After immobilisation of one hindlimb the changes in
flow observed were bilateral, and thus arising from general
factors rather than from the immobilisation itself. These
changes were not statistically significant with the numbers
of animals studied. The observed rise in marrow flow
between one and two weeks may be related to the
haematopoietic function of the marrow in the replacement of
the withdrawn blood cells, particularly as it was more
marked in the proximal marrow which remains macroscopically
red in the adult animal. The increase in proximal marrow
flow between one and two weeks of immobilisation was the
only statistically significant change observed.

The findings of this quantitative study contrast with those of perfusion experiments, which suggest an increase in the vascularity of an immobilised limb within the time periods of the present investigation. The visualisation of blood vessels by perfusion techniques gives only an indirect indication of the level of blood flow, and the vessel proliferation observed around the bones does not appear to be associated with unilateral changes in flow within the tibial diaphysis.

The importance of these findings in the present study is that the unfractured tibia may be used as a valid control for blood flow measurements in a tibia subjected to a unilateral experimental fracture immobilised in a cast. Control measurements of blood flow before fracture may be omitted, allowing more animals to be studied within the radio-isotope disposal limits. The groups of animals studied in this experiment form control groups for future work, allowing assessment of the effects of fracture on blood flow independently of any effects of immobilisation *per se*, or of the effects of operative trauma, although the trauma of an experimental fracture may not be strictly comparable to that of catheterisation for blood flow measurement.

Table 2.5.1 Leaching of Gd^{153} and Sn^{113} labels into the urine and faeces over two weeks (n=1). Leaching is expressed as a percentage of the administered dose. No faeces were passed on the days left blank.

	Urine		Faeces	
Day	Gd^{153}	Sn^{113}	Gd^{153}	Sn^{113}
1	<0.01	<0.01	–	–
2	<0.01	<0.01	–	–
3	0.01	0.01	<0.01	<0.01
4	0.02	<0.01	–	–
5	0.01	<0.01	<0.01	<0.01
6	0.02	<0.01	0.02	<0.01
7	0.06	<0.01	<0.01	<0.01
8	0.07	<0.01	0.01	<0.01
9	0.11	<0.01	<0.01	<0.01
10	0.03	<0.01	–	–
11	<0.01	<0.01	<0.01	<0.01
12	<0.01	<0.01	<0.01	<0.01
13	<0.01	<0.01	<0.01	<0.01
14	0.07	<0.01	<0.01	<0.01
Sum	0.42	0.03	0.04	0.02

Table 2.5.2   One week group.   Blood flows to the tibial diaphysis and the muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres before and after immobilisation of one hindlimb in a cast for one week. Flows are expressed as mean ± SE ml/min.100g (n=6).

		Before cast			After cast			p
proximal cortex	E	1.39	±	0.52	0.59	±	0.16	NS
	C	2.01		0.73	0.77		0.30	NS
distal cortex	E	1.56		0.56	0.61		0.13	NS
	C	1.21		0.41	0.54		0.15	NS
proximal marrow	E	28.18		6.69	17.58		2.49	NS
	C	27.13		6.37	17.28		2.17	NS
distal marrow	E	4.98		2.04	4.96		2.00	NS
	C	4.53		1.95	3.98		1.60	NS
skeletal muscle	E	6.06		1.32	7.69		1.15	NS
	C	4.80		0.90	5.85		0.52	NS

E = experimental limb   C = control limb (not in cast)

NS = not significant at 5% level (paired t test)

Table 2.5.3   Two week group.   Blood flows to the tibial diaphysis and the muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres before and after immobilisation of one hindlimb in a cast for two weeks. Flows are expressed as mean ± SE ml/min.100g (n=6).

		Before cast		After cast		p
proximal cortex	E	1.53	± 0.39	1.36	± 0.39	NS
	C	1.74	0.31	1.40	0.40	NS
distal cortex	E	1.62	0.41	1.40	0.53	NS
	C	1.50	0.45	0.97	0.21	NS
proximal marrow	E	23.44	3.37	33.37	5.80	NS
	C	24.03	2.52	32.60	5.97	NS
distal marrow	E	6.20	1.41	8.11	1.33	NS
	C	5.12	0.91	6.62	1.21	NS
skeletal muscle	E	7.33	1.68	8.66	2.34	NS
	C	6.25	1.01	6.78	1.40	NS

E = experimental limb   C = control limb (not in cast)

NS = not significant at 5% level (paired t test)

Figure 2.5.1   One week group.   Geometric means of the
ratios between flows in the experimental and control limbs,
before and after immobilisation of one hindlimb in a cast
for one week (n=6 for each subgroup).

Geometric means of ratios
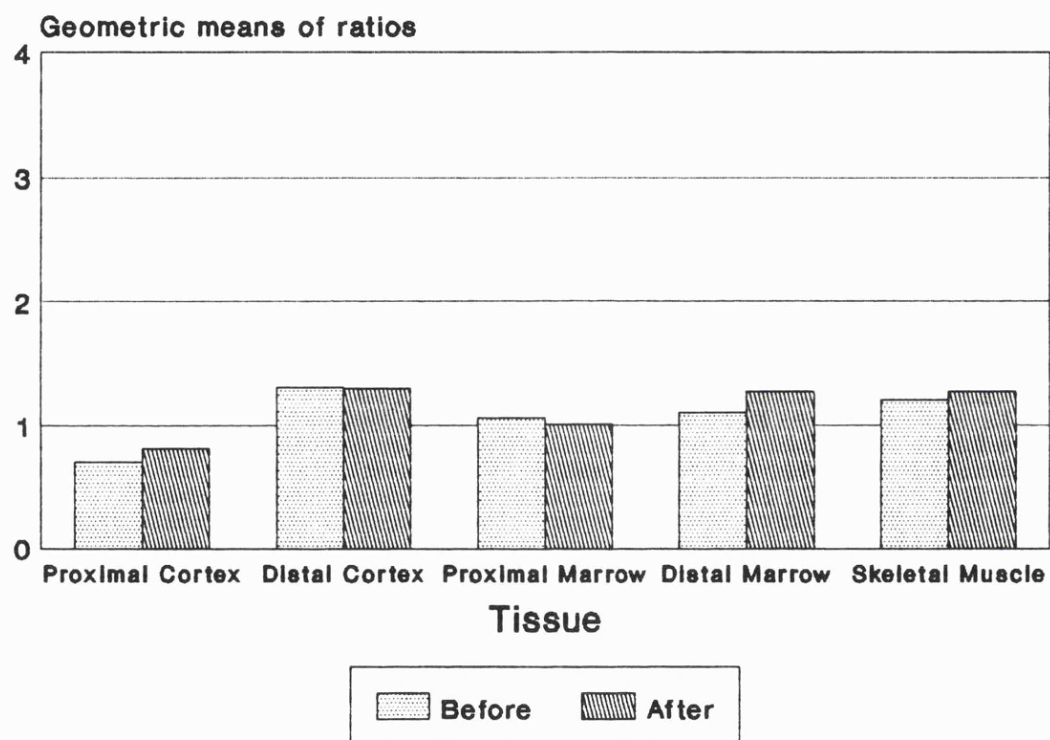
Figure 2.5.2   Two week group.   Geometric means of the
ratios between flows in the experimental and control limbs,
before and after immobilisation of one hindlimb in a cast
for two weeks (n=6 for each subgroup).
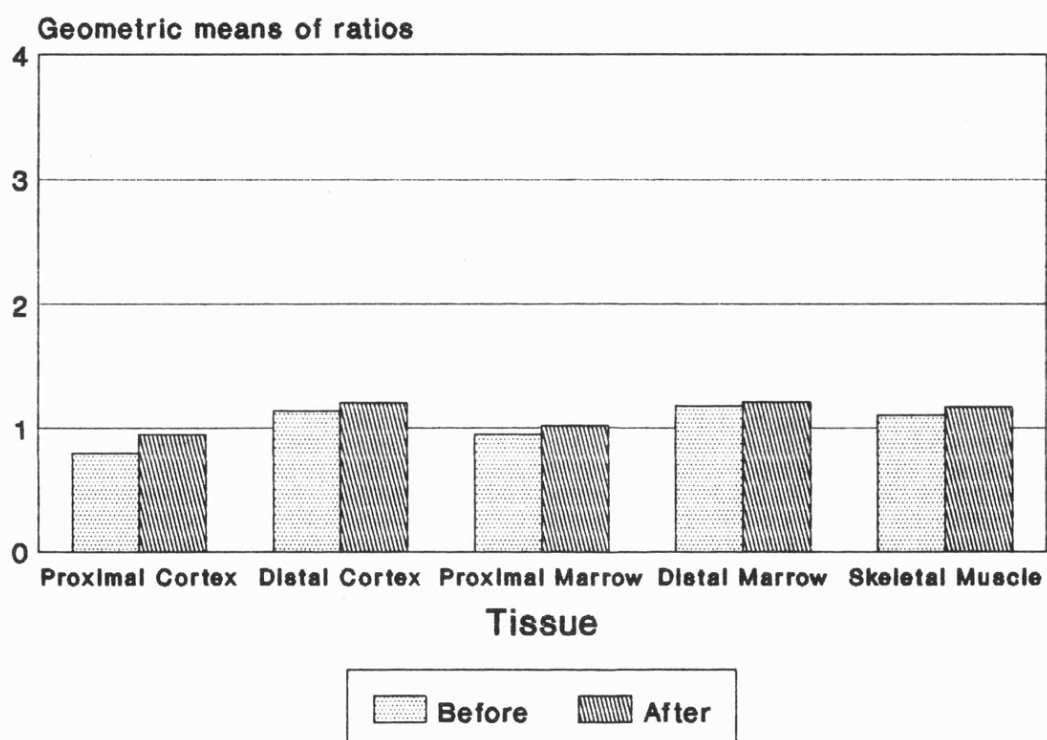
2.6

SUMMARY

For reasons of size and cost, the rabbit has been chosen as the experimental animal for these studies, and as far as possible young adult animals have been used in order to reduce the variation in bone blood flow between animals that is observed in animals of differing age. Anaesthesia was provided by the use of halothane with small doses of opiate, which avoided significant peripheral effects and in particular the opening of arteriovenous anastomoses. It was shown in this animal model that the emissions from two isotopes used to label microspheres could be accurately separated in samples of the tibial diaphysis and skeletal muscle, subject to the requirement of dissolving the cortical samples in acid in order to overcome the inaccuracy arising from their special geometry.

For each model in which the microsphere method is to be used, the microspheres should be adequately mixed at the site of injection. This was tested by comparing the flows to the tibial diaphyses of the two hindlimbs, which were found not to differ. For the microspheres to be evenly distributed within major arteries, and distributed within tissues as nearly as possible in the same manner as erythrocytes, their size should be close to that of erythrocytes while avoiding capillary non-entrapment. Soft tissue studies by other workers have indicated the optimum size to be 10μm or slightly above, but the diffuse venous drainage of bone presents difficulties in the assessment of non-entrapment in this tissue. Such an assessment is limited to comparison of calculated flows obtained with microspheres of two sizes injected simultaneously, if the whole of the circulation of the diaphysis is to be

investigated non-invasively without artefactual non-entrapment from non-osseous tissues.

It is generally believed that microspheres nominally sized 15μm are the smallest that can be used in bone without significant capillary non-entrapment. However, it was found that microspheres sized 16.5 ± 0.1 μm (SD) and 11.3 ± 0.1 μm injected simultaneously gave calculated flows in tibial diaphyseal cortex and marrow, and in skeletal muscle, that were not significantly different. The calculated cortical flows were higher with the smaller spheres, possibly as a result of reduced steric restriction in the small cortical vessels, but this effect was not statistically significant. As smaller microspheres are more evenly mixed in blood vessels, less likely to give rise to central side-effects in large dosage, and considerably cheaper, they were used for the remainder of the study.

One advantage of the microsphere method is the ability to measure flow on more than one occasion without surgical interference with the bone. This requires that the embolisation of small vessels by one injection of micro-spheres does not reduce flow sufficiently to interfere with a subsequent blood flow measurement. It has been found that a range of dosage of up to 1.8×10^6 microspheres per kilogram body weight did not show any tendency to reduce subsequent tibial diaphyseal and skeletal muscle blood flow, and doses of microspheres were kept within this range in the remaining experiments.

Sequential measurements of blood flow were then used to quantify the early effects of cast immobilisation of one hindlimb on tibial diaphyseal blood flow. A pilot experiment indicated that leaching of isotope from the microspheres *in vivo* was negligible. No differences were found between the experimental and control limbs, although flow to the proximal marrow increased significantly between one and two weeks in both limbs. This change may have reflected increased haematopoietic activity. This study, the first of its kind, demonstrated that the opposite limb

could be used as a control during the investigation of blood flow to the tibia after a unilateral experimental fracture immobilised in a cast, allowing a reduction in the variation between animals during statistical analysis.

PART 3

EXPERIMENTAL TIBIAL FRACTURES

3.1 —————————————————————————————————————

A QUANTITATIVE STUDY IN THE ADULT RABBIT OF THE EFFECTS OF
MARROW OR SOFT TISSUE EXCLUSION ON BLOOD FLOW TO THE TIBIAL
DIAPHYSIS AFTER AN OSTEOTOMY

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3.1.1 INTRODUCTION

The results of perfusion studies employing tissue exclusion
techniques suggest that the supply from the periosteum and
soft tissues is more important than that from the marrow in
long bone fracture healing (Trueta and Cavadias 1955,
Göthman 1960b,1961, Cavadias and Trueta 1965, Oni 1987,
Section 1.3.1). However, only the exclusion of the marrow
circulation has been the subject of quantitative studies,
pari passu with investigation of flow after intramedullary
nailing.

This technique of fracture management involves the
removal of a variable amount of medullary tissue and inner
cortex, with their replacement by a rod. The medullary
circulation is destroyed by this procedure, and depending
on the available space between the rod and the cortex, the
regrowth of the marrow vessels is prevented to a variable
degree (Rhinelander 1968, Kessler *et al.* 1986). Cortical
blood flow at the site of an experimental osteotomy treated
in this way has been measured in the canine tibia and ulna
(Table 1.3.1).

Barron *et al.* (1977) compared nailed osteotomies of the
ulna with osteotomies left untreated. Between one and
ninety days postoperatively there were no significant
differences in flow to the fracture site as measured by
strontium clearance. Rand *et al.* (1981) performed a similar
study in the canine tibia and found strontium clearance at
the fracture site to be significantly higher in nailed

osteotomies than in plated bones at forty-two and ninety days. Smith *et al*. (1987), using the microsphere technique, found cortical flows at the fracture site not to differ significantly between plated, externally fixed or nailed osteotomies of the canine tibia at fourteen days. These workers claim to have separated 'endosteal' from 'periosteal' cortex, and their finding was that at this time nailing had reduced flow to the inner cortex with respect to the externally fixed limbs. Differences from the results of Rand *et al*. were attributed to the inclusion in the cortical samples by the latter of the exuberant callus that forms around nailed osteotomies. These studies support the concept that the principal route of blood supply during the peak of the vascular response in the early stages of fracture healing is from the periosteum and soft tissues. However, the quantitative effects of the exclusion of these tissues have not been tested.

A study was therefore carried out in order to establish any information that exclusion methods can give as to the relative capacities of the soft tissue and marrow circulations in the mounting of the blood flow response after fracture of the tibial diaphysis. The experimental model chosen has been investigated previously by a vascular perfusion technique (Oni 1987). A silastic sheath is used to exclude the periosteum and soft tissues from the site of the osteotomy, while the nutrient circulation is excluded by the insertion of an intramedullary nail.

Perfusion studies performed immediately *post mortem* (Oni 1987) have shown that the anastomoses between the epiphyseo-metaphyseal supply and the nutrient circulation are sufficient to allow filling of the medullary vessels of the distal fragment after an osteotomy. The subperiosteal insertion of a sheath around the osteotomy did not prevent this filling, but after manual removal of the bulk of the marrow and the insertion of an intramedullary nail the filling on both sides of the osteotomy was greatly diminished, although not abolished. At one week, some vessels could be filled in the marrow of the distal

fragment of the sheathed osteotomies, but this was not possible at two weeks. At both time periods proliferation of local soft tissue and periosteal vessels was apparent in the nailed osteotomies, with greater than usual penetration of the cortex by these vessels. It was concluded from these studies that the periosteal and soft tissue blood supplies were the more important at this stage of fracture healing, which corresponds to the time of the height of the quantitative blood flow response (Section 1.3.2)

A potential problem of the use of intramedullary nailing as a method of excluding the marrow circulation is the forcible expulsion of marrow fat into the cortex. During insertion of instruments into the medullary canal of the intact rabbit tibia, intramedullary pressures may momentarily exceed 300mmHg, and reaming of the cortex with a powered burr results in a variable loss of cortical perfusion, as assessed by Indian ink infusion, associated with microscopic fat embolisation of the Haversian canals (Danckwardt-Lillieström 1969). Suction of the canal through a cortical window during reaming reduces the apparently avascular area of the cortex (Danckwardt-Lillieström et al. 1970). In the present study, diaphyseal marrow was removed by manual scraping in order to reduce fat extrusion while the extra trauma of a cortical window was avoided.

3.1.2 METHOD

The study was performed on a total of 36 adult female New Zealand White rabbits weighing 3.30 to 5.50kg.

3.1.2.1 *Tibial osteotomy*

General anaesthesia was induced and maintained as in the previous studies, and one limb chosen at random by the toss of a coin. This limb was shaved from the hock to the groin, and 1ml of 2% lignocaine injected percutaneously around the proposed site of the osteotomy. This manœuvre provided

additional analgesia both during and after the procedure. Under aseptic conditions an incision was made along the anteromedial border of the tibia, and the shaft approached posteriorly to the extensor hallucis longus muscle. The soft tissues were raised extraperiosteally over a distance of approximately 1cm, and protected by MacDonald dissectors passed around the shaft. Under saline irrigation a transverse osteotomy was then performed at the mid-point between the distal end of the tibial tuberosity and the medial malleolus, using a reciprocating microsaw (Amsco Hall Surgical, Santa Barbara, USA, distributed by Zimmer, Swindon). The osteotomy site was the same as that used to divide the diaphysis during the preparation of the bone samples from the previous studies. The animals were divided into three groups, with twelve animals in each group :

1. Sheath group. The periosteum with its attached soft tissue was elevated for 1.5cm either side of the osteotomy, which was then surrounded by a 3cm length of silastic tubing opened along one side. This sheath was held in place by a cerclage wire both proximally and distally. The proximal limit of the periosteal stripping was always distal to the distal tibiofibular syndesmosis, and therefore damage to the extra-osseous parts of the nutrient vessels was avoided.

2. Nail group. As much as possible of the marrow was scraped out, and the largest nail selected that would enter the intramedullary canal without the use of reaming. These nails comprised Kirschner wires, Rush pins and orthopaedic guide wires cut to the appropriate length. The nail was inserted into the proximal fragment from the osteotomy site. After flexion of the knee, the nail was passed through the upper end of the tibia anterior to the joint surface, and through a stab incision made in the overlying skin. It was then driven down into the distal fragment after reduction of the osteotomy. In no case did the nail fit tightly enough to prevent rotation at the site of the osteotomy.

3. Control group. No further procedure was carried out in the animals in this group (Figure 3.1.1).

After instillation into the wound of 0.25ml of *Duplocillin* (procaine and benzathine penicillins, Mycofarm UK Ltd, Cambridge), the deep fascia and skin were closed with 4/0 coated polyglactin (*Vicryl*, Ethicon, Edinburgh). A cast was applied in all cases as in Section 2.5, and lateral radiographs were taken of the osteotomies in the control group. It was assumed that the osteotomies in the other two groups remained in apposition at this stage, as previously found (Oni 1987). Postoperative analgesia was supplemented by intramuscular buprenorphine (Reckitt and Colman, Hull), and on the second or third day the animals were returned to the free-range room.

3.1.2.2 *Blood flow measurement*

Six animals in each group were studied one week after osteotomy, and the remaining six after two weeks. Paired observations at these time periods were not undertaken as this would have entailed three operative procedures in two weeks for each animal. Measurement of blood flow to the tibial diaphysis and skeletal muscle was performed as previously described (Section 2.2.2) using $5.0-5.2 \times 10^6$ microspheres sized 11.3 ± 0.1 µm and labelled with Sn^{113}. In six cases catheterisation of the brachial artery failed to provide ready withdrawal of blood, and therefore the withdrawal sample was taken from the right carotid artery. After completion of blood flow measurement, a lateral radiograph was made of the experimental limb before removal of the cast.

3.1.2.3 *Sample preparation*

The tissue specimens were prepared as in previous studies (Sections 2.2.2.3 and 2.2.5.1) using the osteotomy as the division of the diaphysis into proximal and distal parts. The control diaphysis was divided at the same level as the

osteotomy of the experimental bone. Any callus that could
not be removed from the cortex by scraping with a blunt
scalpel was left to be included in the cortical samples.
Marrow remnants were found in all the nailed tibiæ, owing
to the difficulty posed by the narrow diameter of the
medullary canal in scraping out this tissue through a
single osteotomy, and these were removed and counted in the
usual way.

3.1.2.4 *Analysis*

Flows to the two limbs were compared using the paired t
test, and flows in the one and two week subgroups by the
unpaired t test.

Comparisons between the three experimental groups and
with the results of cast immobilisation alone (Section 2.5)
were made by comparing the ratios of the flows to the
experimental and control limbs. The null hypothesis is that
the ratio of the ratios from two different groups is unity.
This hypothesis was therefore tested by applying the
unpaired t test to the logarithms of the ratios.

3.1.3 RESULTS

As found after cast immobilisation without osteotomy, the
animals lost weight over the first two or three days post-
operatively, although in four cases this continued for
between seven and eleven days. In three cases reapplication
of the cast was required after the development of
loosening, swelling of the foot, or reduction of the cast
by gnawing. There were no clinical infections. None of the
osteotomies was clinically united at the time of flow
measurement. The results are summarised in Tables 3.1.1 to
3.1.4, and the full results are given in Tables A.3.8 to
A.3.13 in Appendix 3. Table 3.1.4 is presented graphically
in Figures 3.1.2. and 3.1.3.

It should be noted that the geometric means of the experimental to control ratios given in Table 3.1.4 are not equivalent to the ratios of the mean flows given in the first three tables. The former calculation probably gives a more accurate picture of the relation of the experimental to control limbs in these small data sets, but where the differences between the limbs are not statistically significant no firm conclusion can be drawn as to which limb had on average the greater or lesser flow.

The number of microspheres in each sample exceeded 150 in 89.4% of cases.

3.1.3.1 *Control group*

All but two of the control osteotomies displaced while in the casts, resulting in shortening of up to 15mm (Figure 3.1.4). Radiological callus was observed in none of the one week subgroup, and in two of the two week subgroup.

At one week, the flows to the tissues of the experimental limb were not significantly different to those of the control limb, except for those to the proximal cortex which were higher on the side of the osteotomy (Table 3.1.1). At two weeks, flows to the distal cortex and skeletal muscle were also significantly raised in the experimental limb. Flows to the control limb did not differ significantly between the one and two week subgroups, while in the experimental limb the flows in the two week subgroup were significantly higher to the cortex and distal marrow than at one week.

Whenever flow was significantly increased in the experimental limb, the experimental to control ratio was also significantly higher than that found in the corresponding subgroup of the cast immobilisation study (Table 3.1.4).

3.1.3.2 *Sheath group*

All of the sheaths remained in place, but in three cases there was overriding of the fragments within the sheath of up to 6mm. In three of the two week subgroup there was radiological callus at either end of the sheath (Figure 3.1.5). Extra-osseous tissue did not reach the bone through the potential gap along the sheath, and the limitation of the dissection at the distal tibiofibular syndesmosis prevented damage to the nutrient vessels.

Only in the skeletal muscle samples was there any significant difference between the flows to the two limbs (Table 3.1.2), and this comprised a higher flow in the experimental limb in the two week subgroup. Flows to the distal marrow of both limbs was significantly higher at two weeks than at one week, while in the proximal marrow this effect was significant on the control side only. However, there were no significant differences found on comparison with the cast immobilisation subgroups (Table 3.1.4).

3.1.3.3 *Nail group*

The intramedullary nails prevented displacement and over-riding of the fragments. Radiological callus was visible in one of the one week subgroup and in all of the two week subgroup (Figure 3.1.6).

At one week the flow to the proximal marrow remnants of the experimental tibia was significantly lower than that to the control bone. At two weeks, this drop was more marked, while the flows to the distal cortex and skeletal muscle had become significantly higher on the experimental side (Table 3.1.3).

On comparing the experimental to control ratios with those of the cast immobilisation study, at one week the ratios for the proximal and distal marrow were significantly lower in the nail group, while at two weeks this effect was observed only in the proximal marrow. In

the two week subgroup the ratios for the cortical samples
were significantly higher (Table 3.1.4).

3.1.3.4 *Comparisons between experimental groups*

One week. The experimental to control ratio for the
proximal cortex was significantly higher in the control
group than in the sheath group. The ratio for the proximal
marrow was significantly lower in the nail group than in
either of the other two groups.

Two weeks. The ratios for the distal cortex and skeletal
muscle were significantly higher in the control group than
in the sheath group. Again, the ratio for the proximal
marrow was lower in the nail group than in either of the
other two groups.

3.1.4 DISCUSSION

In the control group, the flows to the cortex of the tibial
diaphysis after an osteotomy without internal or external
fixation have been found to increase with respect to the
control limb as a result of the osteotomy, both proximal to
the division of the bone, and distally after a delay of
between one and two weeks. This delay is despite the
presence of anastomoses allowing perfusion of the distal
marrow vessels immediately after an osteotomy (Oni 1987),
but accords with the severe loss of filling of distal
vessels seen on angiograms of displaced tibial osteotomies
held by an external fixator (Brueton *et al.* 1990). Kunze *et
al.* (1981) observed that the drop in cortical flow to the
distal fragment immediately after osteotomy of the canine
tibia was much greater than that proximally, and it appears
that in the rabbit this deficit is not overcome until the
second week.

The flows to the marrow of the experimental limb were
not found to change significantly with respect to that of
the control limb. This implies that the changes in the

cortical flow observed in the experimental limb were mediated by a supply parallel to that to the marrow, either from the periosteum and soft tissue or within the marrow itself.

The abolition of the cortical blood flow changes by the sheath suggests that this parallel supply arises from the soft tissues close to the osteotomy site. In the nail group these tissues were left intact, but the devascularisation of the cortex by the damage to the marrow, which is much more extensive than that found after lifting of the periosteum (Section 1.2.5), reduced the net effect of the increase in extra-osseous supply. Only in the distal fragment after two weeks did the cortical flow exceed that of the control limb in this group. However, at neither time in this group did the experimental to control ratios for the cortex differ significantly from those of the control group, a finding similar to the results of a previous study of intramedullary nailing over a similar time period (Barron et al. 1977).

An explanation for the changes observed may be sought in the exclusion of venous drainage of the bone rather than of the arterial supply. Thus, the effect of the sheath might have been to inhibit the response of the medullary circulation by preventing adequate venous drainage of the cortex. While it is probable that the principal direction of venous drainage of the cortex is usually centrifugal (Section 1.2.1), the connections of the cortical lattice with the medullary sinusoid system, and the apparently large capacity of the veins draining the peripheral marrow and central sinus (Oni et al. 1987, Oni, Stafford and Gregg 1988), make it unlikely that the sheath caused significant venous obstruction, as it covered only about half of the length of the diaphysis.

The absolute marrow flows were low on the nailed side owing to the reduction in the amount of marrow tissue present, but the level of flow maintained in these remnants emphasises the degree of anastomosis within the marrow, fed

both by anastomoses from the epiphyseo-metaphyseal supply and in normal circumstances by the nutrient artery. This had been indicated by *post mortem* perfusion studies after reaming and nailing (Oni 1987). The marrow flows to the sheath group at two weeks did not, however, parallel the reduction in vascularity found in previous perfusion studies (Oni 1987), emphasising that vessel filling by perfusion methods, or its lack, does not necessarily accord with blood flow.

The value of comparison with an unoperated control limb is demonstrated by the differences in flows to the control limb. For convenience, these flows have been summarised in Table 3.1.5. At one week, those to all tissues examined in the nail group were significantly higher on unpaired *t* testing than those in the cast immobilisation group, and similarly those to the four osseous tissues after nailing were significantly higher than in the sheath group. This may have arisen from differences in the ages of the animals in the one week subgroups, as the mean weight in the nail group was 3.98kg while in the immobilisation, sheath and control groups the means were 4.63, 4.23 and 4.16kg respectively. These differences were not statistically significant, but it is known that bone blood flow tends to diminish with increasing age even in mature animals (Section 2.1.1.2). Alternatively, the differences may have arisen from differing systemic effects of the various operative procedures, as the differences persisted only in the distal marrow in the two week subgroup despite the mean weights of the animals having the same trend across the groups (3.84, 4.44, 4.18, and 3.98kg respectively). A control blood flow measurement carried out before any procedure on the limbs, as performed in the cast immobilisation study, would have assisted in the answering of this question, but in view of the limits on microsphere usage imposed by the isotope disposal regulations (Section 2.1.3.4) it had been considered preferable to perform only one measurement in each animal rather than to halve the number of animals in each subgroup. Whatever the mechanism

of the changes in control limb flow, direct comparison of
the nail group with the others at one week would have led
to an underestimation of the significance of the drop in
marrow flow and an overestimation of that of the rises in
cortical flow. This finding casts doubt on the validity of
comparisons based on studies of bilateral osteotomies
treated by different methods.

Neither the sheath nor the nail prevented an increase
in the flow to the skeletal muscle of the experimental limb
at two weeks. This increase probably arose *pari passu* with
the increase in soft tissue supply to the bone, and the
trend in the mean experimental to control ratio across the
groups matches that of the cortical samples (Table 3.1.4).
It was not possible to measure flow to the periosteum
itself, as this tissue could not be isolated from the soft
tissue mass around the healing osteotomies. The small
amount of this tissue would in any event have made
estimation of its blood flow unreliable.

The changes in the flows found in the control group of
this study are generally smaller than those previously
reported after osteotomy with internal or external fixation
(Table 1.3.1). There are two possible reasons for this.
Firstly, most of these former studies used strontium
clearance for the measurement of blood flow, effectively to
the cortex alone, and apart from the other disadvantages of
this method, the extraction of the isotope after the first
minute may be greater in areas of new bone formation, as is
found with the bone scanning agent methylene diphosphonate
(Hughes *et al.* 1978, Nutton *et al.* 1985). However, within
the five or ten minute period of a clearance measurement
the tibial extraction of strontium after nutrient artery
injection does not appear to be changed by osteotomy
(Laurnen and Kelly 1969, Hughes *et al.* 1979).

Secondly, the previous studies were performed on the
dog, in which the segments of diaphysis containing the
osteotomy constituted a smaller part of the total length of
the bone. This may also explain the higher cortical flows

found by Smith *et al.* (1987) using the microsphere
technique in the canine tibia. The use of smaller bone
samples from either side of the osteotomy might have
resulted in higher flows per unit weight in the present
study by excluding bone further away that probably had a
lower flow, but this would have reduced the numbers of
microspheres in each sample and therefore increased
experimental variation.

A major problem with exclusion studies of this kind is
the variation between experimental groups other than in the
change in the type of exclusion. The principal variant is
in the mobility of the osteotomy site during healing. This
parameter did not appear to have a consistent effect on the
tissue flows. The principal effect of movement would
probably be the inhibition of the re-establishment of the
medullary circulation across the osteotomy, but the period
of this study is less than time required for this to occur
in conditions other than rigid immobility (Rhinelander
1968, Rhinelander *et al.* 1968, Holden 1972, Chidgey *et al.*
1986, Brueton *et al.* 1990). The use of internal or external
fixation to control mobility would have added interference
with the bone circulation in addition to that conferred by
the exclusion methods. Brueton *et al.* (1990), using
external fixators, found that loss of contact between the
bone ends after an osteotomy of the rabbit tibia is
associated with a delay in the opening of vessels in the
distal fragment as demonstrated by perfusion studies, but
even in those osteotomies kept under compression the
process of revascularisation extended over a period longer
than that of the present study. Further, the comparison
between the cortical flows of the control and nail groups
in this study is similar to that of Barron *et al.* (1977),
in whose study the control ulnar osteotomy was much more
stable as a result of splinting by the radius.

A second variable is the extent of the exclusion along
the shaft. Intramedullary nailing will effectively result
in damage to the medulla along the entire length of the
diaphysis, while the length of the sheath is limited by the

requirement of avoiding damage to the nutrient vessels. The observed limitation of the blood flow response in the sheath group implies that the sheath was sufficiently long to cover the area of the great majority of soft tissue response.

This study has shown that the blood flow response near the peak of the blood flow changes after osteotomy between one and two weeks is mediated principally by the periosteum and soft tissue. This confirms the suggestion of Rhinelander in his qualitative description of the early changes in fracture site vascularity (Rhinelander 1974), and the recruitment of soft tissue supply at this time observed after nutrient artery occlusion observed by Strachan et al. (1990). These workers observed that two weeks after osteotomy of the canine tibia, occlusion of the nutrient artery caused a much reduced fall in diaphyseal flow than that found without osteotomy. This experimental method constitutes a type of exclusion, albeit delayed until some stage during healing, which indicates the amount of blood supply that is obtained only from the tissue excluded without the background of the facultative development of other routes of supply.

While this method of 'secondary' exclusion might appear more physiological, the osteotomy was still subject to a degree of 'primary' exclusion in the form of the fixation plate. It also required the interference of surgical exposure of the bone at the time of blood flow measurement. Division of the nutrient artery requires less surgery than elevation of the periosteum and soft tissue from the fracture spindle, which is difficult to perform with any accuracy in view of the lack of clear tissue planes. To allow medullary reaming, initial operative fixation would be required to prevent overriding of the fragments.

Primary exclusion methods employ surgical trauma at the time of the experimental fracture, and thus more closely mimic the potential tissue damage that may occur in clinical injuries. However, the continuance of exclusion

during healing does not match the clinical course of
fracture healing unless the technique is one used in
fracture management, as with intramedullary nailing. The
methods used in this study were selected in view of the
previous studies by perfusion techniques, but a more
satisfactory alternative might have been the use of soft
tissue stripping and medullary reaming without the use of
the sheath or nail. Thus, standard types of tissue damage
would have been produced, while allowing uninhibited
recovery from this damage. The problem of the variable
amount of fracture mobility between the three groups would
also have been avoided. Such a model would still be far
from identical to a clinical fracture, and an alternative,
based on fractures produced by blunt trauma, is the subject
of the next study.

Table 3.1.1   Control group.   Blood flows to the tibial
diaphysis and the muscle of the anterior compartment,
measured with 11.3 ± 0.1 (SD) μm microspheres one and two
weeks after a unilateral tibial diaphyseal osteotomy. Flows
are expressed as mean ± SE ml/min.100g   (n=6 for each
subgroup).

		Control		Osteotomy		p
proximal cortex	1	1.42 ± 0.34		2.76 ± 0.50		<0.01
	2	2.01	0.54	5.89*	1.09	<0.01
distal cortex	1	1.83	0.59	1.79	0.36	NS
	2	1.99	0.56	5.85**	0.94	<0.001
proximal marrow	1	26.96	7.46	32.44	8.82	NS
	2	34.13	6.58	28.08	3.47	NS
distal marrow	1	9.08	2.23	8.64	1.66	NS
	2	14.79	4.63	13.74*	1.41	NS
skeletal muscle	1	10.16	3.14	11.28	2.03	NS
	2	6.49	1.41	13.12	2.45	<0.01

Control = control limb (no osteotomy)

Osteotomy = experimental limb

1 = one week subgroup

2 = two week subgroup

NS = not significant at 5% level (paired t test)

 * = significantly different from one week flow at 5% level
        (unpaired t test)

** = significantly different from one week flow at 1% level
        (unpaired t test)

Table 3.1.2   Sheath group.   Blood flows to the tibial
diaphysis and the muscle of the anterior compartment,
measured with 11.3 ± 0.1 (SD) μm microspheres one and two
weeks after a unilateral tibial diaphyseal osteotomy with
the periosteum and soft tissues excluded by a silastic
sheath. Flows are expressed as mean ± SE ml/min.100g (n=6
for each subgroup).

		Control		Osteotomy		p
proximal cortex	1	1.08 ± 0.42		1.28 ± 0.49		NS
	2	2.00	0.88	2.20	0.37	NS
distal cortex	1	0.75	0.27	0.89	0.31	NS
	2	1.80	0.94	1.87	0.40	NS
proximal marrow	1	17.57	3.80	16.39	3.80	NS
	2	32.13*	5.15	28.04	4.47	NS
distal marrow	1	4.62	1.24	4.98	1.27	NS
	2	12.49**	2.03	12.92*	3.05	NS
skeletal muscle	1	6.18	2.66	7.57	2.50	NS
•	2	8.92	3.35	11.91	3.91	<0.05

Control = control limb (no osteotomy)

Osteotomy = experimental limb

1 = one week subgroup

2 = two week subgroup

NS = not significant at 5% level (paired t test)

• = significantly different from one week flow at 5% level

     (unpaired t test)

•• = significantly different from one week flow at 1% level

     (unpaired t test)

Table 3.1.3   **Nail group.**   Blood flows to the tibial diaphysis and the muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres one and two weeks after a unilateral tibial diaphyseal osteotomy with intramedullary nailing. Flows are expressed as mean ± SE ml/min.100g (n=6 for each subgroup).

		Control		Osteotomy		p
proximal cortex	1	2.50	± 0.47	3.85	± 1.09	NS
	2	1.45	0.25	2.72	0.71	NS
distal cortex	1	3.57	0.86	5.55	1.75	NS
	2	1.79	0.59	4.02	1.01	<0.05
proximal marrow	1	59.15	15.32	30.78	11.33	<0.01
	2	48.42	10.05	17.86	10.15	<0.001
distal marrow	1	21.36	6.15	18.23	9.61	NS
	2	17.83	4.26	20.64	11.72	NS
skeletal muscle	1	14.74	3.44	15.14	1.93	NS
	2	7.88	2.46	11.50	3.54	<0.05

Control = control limb (no osteotomy)

Osteotomy = experimental limb

1 = one week subgroup

2 = two week subgroup

NS = not significant at 5% level (paired *t* test)

No two week flow differed significantly from corresponding one week flow (unpaired *t* test)

Table 3.1.4 Geometric means of the ratios between flows in the experimental and control limbs (n=6 for each subgroup).

		Cast	Control	Sheath	Nail
proximal cortex	1	0.81	2.05**	1.17	1.28
	2	0.95	3.17**	1.59	1.73
distal cortex	1	1.30	1.15	1.25	1.24
	2	1.20	3.41**	1.35	2.72*
proximal marrow	1	1.01	1.20	0.94	0.42**
	2	1.02	0.88	0.87	0.15*
distal marrow	1	1.27	1.02	1.06	0.55
	2	1.21	1.15	0.94	0.72
skeletal muscle	1	1.27	1.36	1.27	1.12
	2	1.17	2.14**	1.46**	1.51*

Cast = cast immobilisation only (Section 2.5)

Control = osteotomy with cast

Sheath = osteotomy with silastic sheath and cast

Nail = osteotomy with intramedullary nail and cast

1 = one week subgroups

2 = two week subgroups

* = significantly different from unity at 5% level (paired t test)

** = significantly different from unity at 1% level (paired t test)

Table 3.1.5 Mean flows to the control limbs, expressed as
ml/min.100g (n=6 for each subgroup).

		Cast	Control	Sheath	Nail
proximal cortex	1	0.77	1.42	1.08	2.50
	2	1.40	2.01	2.00	1.45
distal cortex	1	0.54	1.83	0.75	3.57
	2	0.97	1.99	1.80	1.79
proximal marrow	1	17.28	29.96	17.57	59.15
	2	32.60	34.13	32.13	48.42
distal marrow	1	3.98	9.08	4.62	21.36
	2	6.62	14.79	12.49	17.83
skeletal muscle	1	5.85	10.16	6.18	14.74
	2	6.78	6.49	8.92	7.88

Cast = cast immobilisation only (Section 2.5)

Control = osteotomy with cast

Sheath = osteotomy with silastic sheath and cast

Nail = osteotomy with intramedullary nail and cast

1 = one week subgroups

2 = two week subgroups

(a) control   (b) sheath   (c) nail
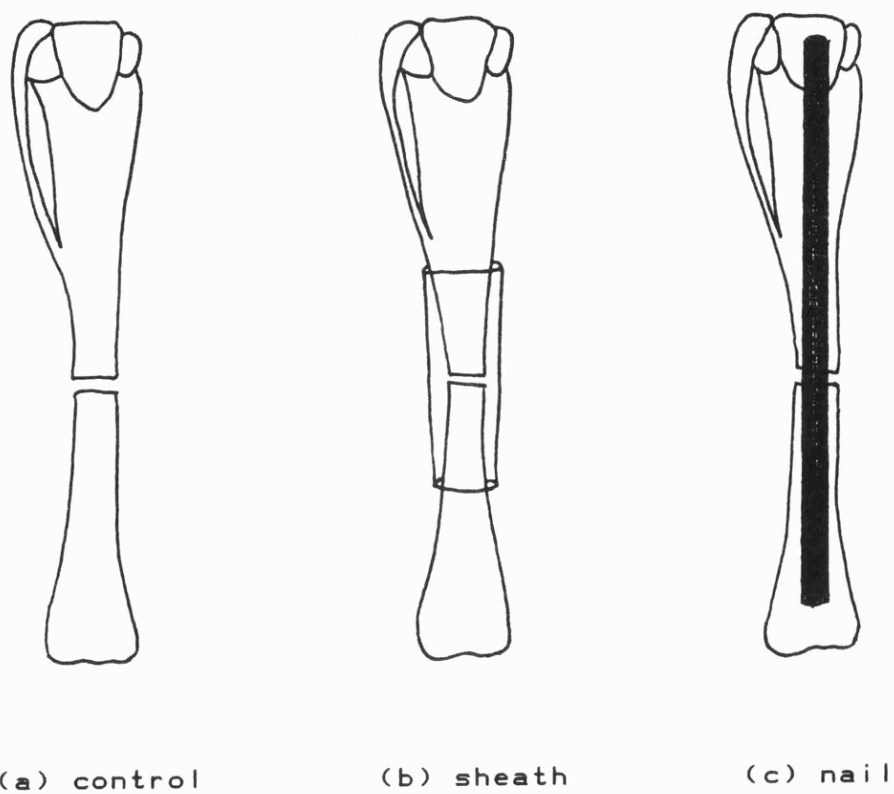
Figure 3.1.1 Experimental osteotomies of the diaphysis of
              the adult rabbit (after Oni 1987).

Figure 3.1.2   One week subgroups   Geometric means of the ratios between flows in the experimental and control limbs at one week   (n=6 for each subgroup).
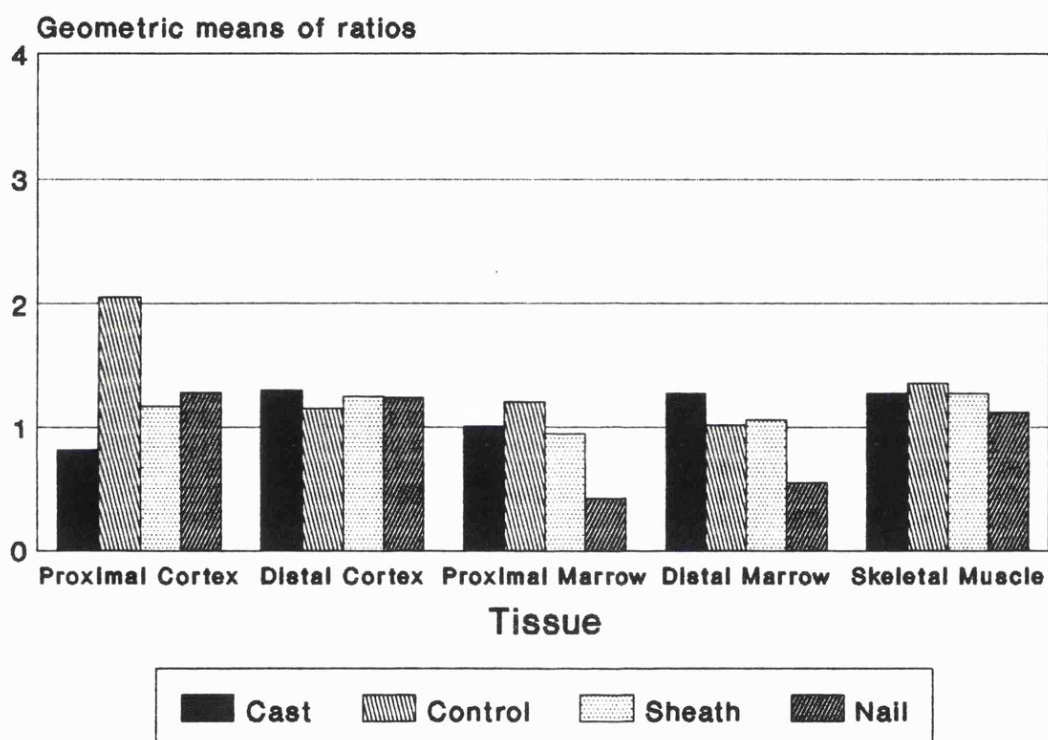
Figure 3.1.3   Two week subgroups   Geometric means of the ratios between flows in the experimental and control limbs at two weeks   (n=6 for each subgroup).

Geometric means of ratios
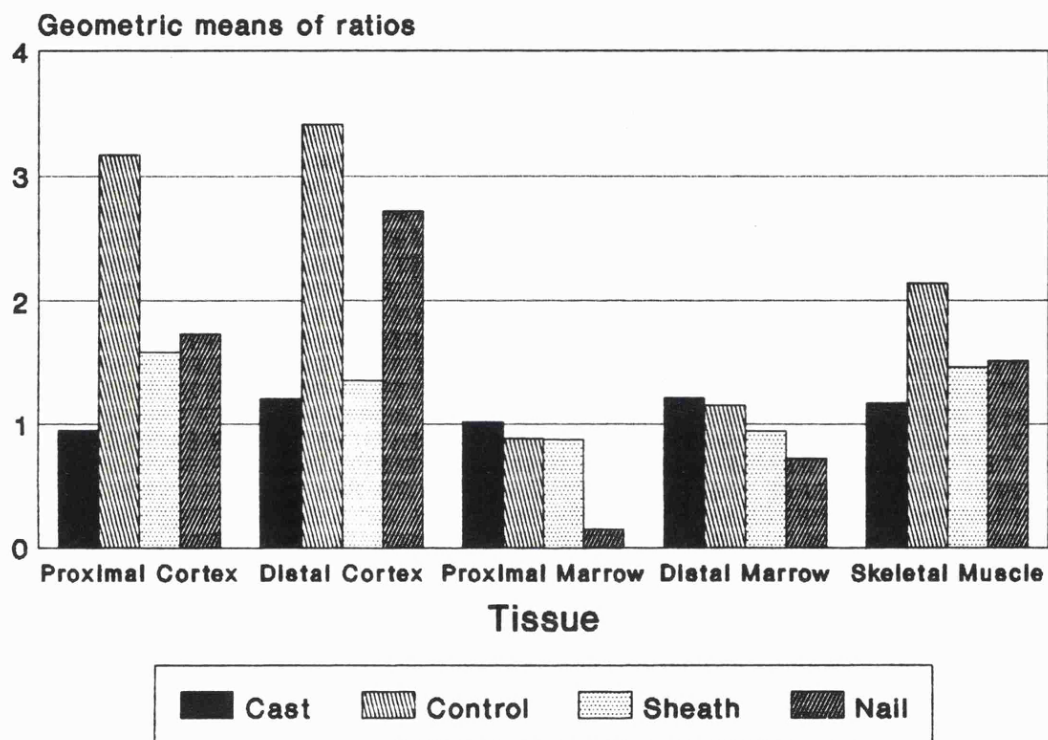
Figure 3.1.4

Control group.
Radiographs of the tibial
diaphysis, one and two
weeks after an osteotomy
in the control group.

(a) one week

(b) two weeks

Figure 3.1.5

Sheath group.
Radiographs of the tibial
diaphysis, one and two
weeks after an osteotomy
in the sheath group.

(a) one week

(b) two weeks

Figure 3.1.6
Nail group.
Radiographs of the tibial
diaphysis, one and two
weeks after an osteotomy
in the nail group.

(a) one week

(b) two weeks

3.2

A STUDY OF THE BLOOD FLOW TO THE TIBIAL DIAPHYSIS OF THE ADULT RABBIT AFTER FRACTURES CAUSED BY DIFFERING FORCES

3.2.1 INTRODUCTION

The great majority of investigations into the blood supply of bone after 'fracture' have been carried out on surgical osteotomies. These may bear little resemblance to fractures caused by blunt trauma, particularly with regard to the associated soft tissue damage. Methods of production of fractures by closed means *in vivo* have included simple manual bending (Wray and Lynch 1959, Wray 1963), bending by hydraulic press with or without manual displacement of the fragments (Rhinelander and Baragry 1962, Rhinelander *et al*. 1968), and percussion by a weight falling under gravity (Edwards 1965b). In no case has the fracture so produced been subsequently studied by quantitative methods, and no attempt has been made to relate bone blood flow to the force used to create the fracture.

The principle determinant of delayed union of clinical fractures of the adult tibial shaft appears to be the energy of the injury (Section 1.1). Thus, closed injuries sustained in vehicular accidents and by falls from a height are more likely to be subject to delayed union than sports injuries and those sustained by lesser falls (Oni, Hui and Gregg 1988), and the features of fractures observed to be prone to delayed union are those commonly resulting from high energy accidents. The analysis of clinical series in Section 1.1.2 suggests that these features are comminution, initial displacement, associated fibular fracture, and compounding, the latter three factors being closely related. The degree of all these parameters is related to

the energy involved in the production of the fracture, and the possible relationship of this energy to the degree of devascularisation of the bone is the basis for the hypothesis to be tested in the present study. Thus it is proposed that tibial fractures resulting from higher energy blunt trauma are associated with an attenuation of the subsequent bone blood flow response (Section 1.1.3).

Higher energy injuries are usually of the bending type rather than the spiral, and are often caused by direct violence to the site of fracture. It is therefore this type of fracture that should be studied first in the assessment of the effect of the energy imparted to the bone on the subsequent blood flow. Such a fracture can be produced by percussion of the shaft.

Using a percussion technique, Edwards (1965b) studied the importance of skin damage on subsequent fracture healing in the dog. The skin over the anterior surface of the control tibia was incised and retracted to expose the bone, and fractures of both tibiae produced by dropping onto the limbs a weight running along two vertical bars. The skin incision and the wound resulting from the blow to the experimental limb were then closed and both limbs immobilised in plaster casts. Clinical union occurred within six weeks in all control limbs, while in the experimental limbs healing was delayed beyond this period in 83% of cases. This was attributed to the 75% incidence of skin 'necrosis' in these limbs, often resulting in exposure of the bone although without frank infection. This qualitative study suggests that care is required to avoid skin necrosis in experimental fractures if different experimental groups are to be comparable.

The fractures produced by Edwards were crush injuries, and therefore were extensively comminuted. However, in the study of the effects of fracture energy, a simple fracture pattern is desirable in order to facilitate both the distinction between flows proximal and distal to the fracture, and reproducibility of fracture morphology in

different animals. Transverse fractures are ideal, and
might be produced by supporting the bone at either end to
enable fracturing by three-point bending.

3.2.2 METHOD

The study was performed on a total of 24 adult female New
Zealand White rabbits weighing 3.40 to 4.50kg.

3.2.2.1 *Fracture rig*

The rig used to produce tibial shaft fractures was a
modification of that used by Edwards (1965b) and was
designed and built in conjunction with the Department of
Engineering of the University of Leicester. Into a heavy
base plate were set two parallel vertical bars, along which
ran a weight bearing a rounded point (Figures 3.2.1 and
3.2.2). To produce a fracture by three-point bending, the
tibia was held away from the base plate by supports
positioned approximately 6cm apart around the hock and
knee. These supports were moulded in silicone polymer
(*Flexane 60*, Devcon Ltd, Shannon, Ireland) from a plaster
of Paris replica of the right hindlimb of an adult New
Zealand White rabbit. The hock moulding was circular in
cross-section, and was mounted between two wooden blocks
between which it could be rotated as required (Figures
3.2.1 and 3.2.3). The knee support was held manually,
giving greater freedom of control of the positioning of the
limbs of different animals in the horizontal plane. The
tibia was held approximately 2.5cm from the base plate, and
a hole drilled through the base plate allowed for downwards
displacement of the fracture fragments up to a theoretical
maximum angulation at the fracture site of approximately
80°.

The energy of the weight while falling under the
influence of gravity, ignoring the effects of friction and
air resistance, is equivalent to its potential energy :

potential energy = mgh   (3.1)

where *m* = mass of the weight

      g = acceleration due to gravity

  and *h* = difference in height between the starting and

         finishing positions of the weight.

Thus, the energy imparted to the limb can be varied by changing the initial height of the weight above the limb or by changing the mass of the weight. As the mechanical properties of bone vary with the rate of its deformation (Sammarco *et al.* 1971, Panjabi *et al.* 1973), it is desirable that the velocity of the weight at impact is kept constant with changes in applied energy. An expression for the velocity of the weight is obtainable from the identity of its kinetic energy to its potential energy in these circumstances, the energy of its motion arising solely from its descent under gravity :

kinetic energy = $\frac{1}{2}mv^2$ (3.2)

where *v* = velocity. Therefore :

mgh = $\frac{1}{2}mv^2$

Solving for *v* gives :

v = $\sqrt{(2gh)}$ (3.3)

It is apparent that velocity is independent of mass if friction and air resistance are ignored. Therefore, the initial height of the weight should be kept constant, and changes in energy should be achieved by varying its mass. Maintaining a constant initial height has the further

advantage of reducing increases in friction with higher energies, as it is difficult to hold two bars parallel over an extended distance, and friction will increase with any deviation. Over the small distances and with the small velocity of this experiment, the effects of air resistance were well within the margin of error of the technique.

The energy of the weight calculated at the point of impact is only an estimation of the energy imparted to the limb, as during the fracture the weight will continue to descend and displace the fragments, and some energy will be absorbed by deformation of the support moulds. Accurate measurement of the actual energy depends upon accurate recording of the resulting strain and displacement, the area under a plot of these two parameters giving the work done, or energy expended, on the limb. However, such measurements pose difficulties. Recording strain by a gauge on the weight would require rigid fixation of either end of the bone, which would require the use of heavy transfixing wires or of limb grips imposing unacceptably high pressures on the soft tissues. Limiting the analysis to the bone itself would require the application of a strain gauge directly onto the shaft. These methods would obviate any advantage the technique could otherwise give in the mimicking of a clinical fracture. It was therefore decided that an estimation of the energy of the weight at the point of impact was as close as should be obtained.

This method of fracture production allowed variation in the amount of energy available at impact, while keeping constant those factors independent of this parameter. These were the rate of deformation, assuming this to depend on the velocity of the weight at impact; the area over which the fracturing force was applied; the direction of the force; and the axial preloading of the bone, which was assumed to be negligible in all cases.

Preliminary testing of the apparatus was carried out with isolated bones and hindlimbs of rabbits obtained from the local market. These limbs were from animals of

indeterminate strain, and lacked both the foot and the
distal 1 or 2cm of the tibia, but it was not felt
justifiable to use experimental animals for this purpose.
The isolated bones were found to fracture at much lower
heights than the tibiæ of complete limbs, probably as a
result of the soft tissues absorbing energy by allowing
displacement of the bones within the supports. The mass of
the weight as constructed was 0.959kg, and with this mass
the lowest height at which fractures were produced in five
hindlimbs were 29, 30, 32, 40, and 40cm. In order to
optimise the likelihood of demonstrating differences
between fractures of differing energy, the difference
between the energies should be as great as possible. The
upper limit is determined by the increasing likelihood of
skin breakdown with increasing energy, which is undesirable
if the groups are to remain otherwise identical. The
initial height of the weight was set at 40cm for the *lower
energy* group, in order to keep the energy at impact as low
as possible while reliably producing a fracture. For the
higher energy group the mass of the weight was
approximately doubled to 1.911kg by the addition of lead
weights. This difference between the groups was considered
to be unlikely to cause skin problems while sufficient to
indicate any differences in blood flow. The energy of the
weight at impact in the lower energy group was approx-
imately 3.76Nm (0.959kg × 9.81ms^{-2} × 0.4m, equation 3.1),
and in the higher energy group approximately 7.50Nm, while
the impact velocity of the weight in both groups was
approximately 2.80ms^{-1} (equation 3.3).

3.2.2.2 *Tibial shaft fracture*

The animals were anaesthetised as in previous studies.
Analgesia was supplemented by percutaneous infiltration of
1ml of 2% plain lignocaine behind the tibia at the proposed
site of fracture. No solution was placed anteriorly between
the bone and the skin, in order to reduce the probability
of shearing injury to the skin.

After application of the proximal mould around the
right hindlimb the hock and foot were mounted in the distal
mould and the bolts of the mounting blocks tightened. The
rotation of the distal mould within the blocks was adjusted
until the anteromedial crest of the tibia was uppermost.
This entailed approximately 45° of external rotation of the
limb, and allowed the limb to be struck without the
imposition of muscle between the weight and the bone. This
reduced variability between the fractures of different
animals. The skin was protected by a double layer of cast
padding (*Soffban*, Smith and Nephew, Hull). While a firm
grip was taken manually of the proximal mould, the weight
was raised 40cm above the point where it rested on the limb
and was allowed to fall onto the limb under gravity. The
limbs of the experimental animals were more resistant to
fracture than those used in the preliminary testing of the
rig, and in nine cases, six in the lower energy group, more
than one attempt was required to cause a fracture. However,
in no case was it necessary to increase the mass of the
weight or its initial height in order to produce a
fracture, the only manœvre required being a small change in
the rotation of the limb or of its position in the
horizontal plane.

After fracture the limb was removed from the moulds,
and placed in a padded cast as in previous studies
(Sections 2.5 and 3.1). Lateral radiographs were obtained of
the fracture within the cast. Postoperative analgesia was
supplemented by intramuscular buprenorphine. On the second
or third day the animals were returned to the free-range
room.

3.2.2.3 *Blood flow measurement*

Six animals in each group were studied one week after
fracture, and the remaining six after two weeks. Paired
observations at these time periods were not undertaken as
this would have entailed three operative procedures in two
weeks for each animal. Measurement of blood flow to the

tibial diaphysis and skeletal muscle was performed as
previously described (Section 2.2.2) using 4.8-5.0 × 10⁶
microspheres sized 11.3 ± 0.1 μm and labelled with Sn¹¹³.
In eight cases catheterisation of the brachial artery
failed to provide ready withdrawal of blood, and therefore
the withdrawal sample was taken from the right carotid
artery. After completion of blood flow measurement, a
lateral radiograph was made of the experimental limb before
removal of the cast.

3.2.2.4 *Sample preparation*

The tissue specimens were prepared and counted as in the
previous study (Section 3.1.2.3) using the fracture site as
the division of the diaphysis into proximal and distal
parts. The control diaphysis was divided at the same level
as the fracture of the experimental bone. Additional
cortical fragments arising from any fracture comminution
were included in the sample of the parent fragment. The
fracture was termed *transverse* if its main limb formed an
angle of more than 45° to the long axis of the bone, and
oblique if less.

   In two cases discussed below, a secondary fracture had
occurred at the upper end of the shaft and had displaced in
the cast. In one this was above the upper limit of the bone
samples, and in the other below. The proximal fragment
taken for counting was in these cases shortened so as to
exclude the fracture and at least 1cm of shaft distal to
it, and the control tibia was sampled similarly.

3.2.2.5 *Analysis*

Flows to the two limbs were compared using the paired *t*
test, and flows in the one and two week subgroups by the
unpaired *t* test.

   Comparisons between the experimental groups and with
the results of cast immobilisation alone (Section 2.5) and
of surgical osteotomy (control group of Section 3.1) were

made by comparing the ratios of the flows to the
experimental and control limbs, as described in Section
3.1.2.4.

3.2.3 RESULTS

As in previous studies, the animals lost weight for the
first few days after fracture, and in three cases in the
two week subgroups this continued over the whole study
period. In three cases reapplication of the cast was
required after the development of loosening, swelling of
the foot, or reduction of the cast by gnawing. A tendency
for the animals to drag the fractured limb was observed.
None of the fractures was clinically united at the time of
flow measurement. The results are summarised in Tables
3.2.1 to 3.2.3, and the full results are given in Tables
A.3.14 to A.3.17 in Appendix 3.

   The number of microspheres in each sample exceeded 150
in 97% of cases.

3.2.3.1 *Lower energy group*

In total, 9 transverse and 3 oblique primary fractures were
produced (Figures 3.2.4 and 3.2.5). In five cases minor
comminution occurred, resulting in extra fragments of up to
approximately 3mm in size. In all but one case the
fragments displaced within the cast, similarly to the
control group of the previous study (Section 3.1),
resulting in shortening of up to 12mm. In one animal of
each subgroup the fracture was segmental, with an
additional fracture at the lower limit of the knee support
(Figure 3.2.6). Displacement occurred at both fracture
sites in the animal in the two week subgroup, but in the
other the secondary fracture remained undisplaced on
radiological examination and was not apparent on inspection
at the time of sample preparation. There was no

radiological callus in the one week subgroup, while at two weeks callus was visible in five animals (Figure 3.2.7).

In most cases the skin was punctured from within out as a result of the firm hold of the hock mould on the distal fragment, which resulted in tenting of the skin at the fracture site during the displacement of the proximal fragment. In one case this puncture was closed with a single polyglactin stitch, while in the others the wound was very small and apparent only because of the formation of a drop of blood on the skin surface. At the time of blood flow measurement the skin was intact in all cases.

At one week the flow to the proximal cortex was significantly raised with respect to the control limb, while flows to the proximal and distal marrow were significantly depressed (Table 3.2.1). At two weeks, the flow to both cortical samples was raised, while the marrow flows were now not significantly different to those of the control limb. The skeletal muscle flows to the control limb at both time intervals were significantly lower than those seen after cast immobilisation alone, but their wide variation resulted in the differences between the limbs not reaching statistical significance.

3.2.3.2 *Higher energy group*

In this group there were 11 transverse and 1 oblique fracture, in four cases in association with minor comminution as found in the lower energy group. In three animals undisplaced longitudinal fissure fractures were produced in either or both fragments, as often found at operation clinically. Shortening within the cast of up to 12mm occurred in all but three animals. In three cases of the one week subgroup the fractures were segmental, the secondary fractures comprising two situated proximally at the lower limit of the knee mould, and one distally at the upper limit of the hock mould. Initially all secondary fractures were undisplaced, but one proximal fracture became displaced within the cast before blood flow

measurement. The remaining two fractures were no longer visible radiologically at the time of blood flow measurement nor on direct inspection of the bone during sample preparation. Radiological callus was seen in one animal of the one week subgroup and four of the two week subgroup (Figure 3.2.8).

As in the lower energy group, small skin puncture wounds were common. In one animal of the one week subgroup an area of skin breakdown measuring approximately 1cm across developed over the fracture site. In view of the findings of Edwards (1965b) referred to above this animal was excluded from the analysis. This exclusion from an already small data set made the attainment of statistical significance more difficult, and may explain the lack of significant differences between the experimental and control limbs at one week (Table 3.2.2). Thus, as found in the lower energy group, the cortical flows were on average higher and the marrow flows lower on the experimental side both proximally and distally, but these differences were not statistically significant.

At two weeks, the flow to the distal cortex on the experimental side was significantly higher, and also the flow to the skeletal muscle, albeit by a small amount. The significance of the rise in proximal cortical flow was just outside the 5% level. The marrow flows were not significantly different from those of the control limb.

3.2.3.3 *Comparisons between experimental groups*

The means of the ratios of experimental and control limb flows are given in Table 3.2.3 and Figures 3.2.9 and 3.2.10. It can be seen that by paired testing of the ratios all the cortical flows in the higher energy group were significantly different from unity, in contrast to the analysis of differences given above. This disagreement between the two analyses arises from the small size of the data sets.

There were no significant differences between the two
energy groups at either time interval. In both groups the
cortical ratios were significantly higher than in the cast
immobilisation groups both at one and two weeks proximally,
and at two weeks distally. The marrow ratios were
significantly depressed at one week in both groups except
proximally in the higher energy group, the lack of
statistical significance probably arising from the smaller
number of animals in this group. The only significant
differences from the control group of Section 3.1 were that
the marrow ratios of the lower energy group were lower at
one week.

The fracture site in the present study was on average
approximately 6mm more distal than in previous experiments,
but these comparisons were considered valid as this
distance is equivalent to less than 10% of the length of
the diaphysis as defined in these studies, and the method
of comparison was based on ratios of *paired* limbs divided
at the same level.

3.2.4 DISCUSSION

This first design of a fracture rig was highly successful
in producing fractures by three-point bending with the
minimum of skin problems. Transverse fractures accounted
for 83%, the remaining oblique fractures presumably arising
from tilting of the tibia from the horizontal. Comminution
was minor. However, 21% of fractures were segmental,
presumably as a result of angulatory forces at the limits
of the limb supports. In half of these cases the secondary
fracture remained undisplaced and healed within a week. In
the other half, which displaced in the cast, the middle
fragment was supplied by the nutrient artery, similarly to
the proximal fragment of the simple fractures. They were
sampled away from the secondary fracture site, following
the finding in the sheath group of the previous study
(Section 3.1) that the great majority of blood supply

response to adult diaphyseal fracture in the rabbit appears to take place within 1.5cm of the fracture site. The proximal fragments as analysed were therefore similar to those in other animals, and these animals were therefore included in the analysis.

In some cases more than one attempt was required to produce a fracture, particularly in the lower energy group. While the numbers of animals in each subgroup does not allow statistical analysis of any differences between these animals and the remainder, the application of the force to the subcutaneous border of the tibia ensured that the effects of the repeated trauma would principally be on the skin, and these animals did not show any evidence of increased skin damage at the time of fracture or at the time of blood flow measurement. The similarity between the lower and higher energy groups suggests that there was little effect on subsequent bone and muscle flow.

On average the fracture site was slightly more distal than in the previous study, which led to a disparity between the lengths of the proximal and distal fragments. This might have led to both a comparative reduction in proximal flow per unit weight after fracture and a rise in distal flow with respect to the control group of the exclusion study, as a result of the inclusion of cortex more remote from the fracture site proximally and only cortex close to the fracture distally (Section 3.1.4). This was generally the case proximally (Table 3.2.3), although the differences were not statistically significant. Any such effect would serve to increase the observed differences with the control group proximally and decrease them distally, in which case it may have masked a reduction in the blood flow response to the distal cortex after blunt trauma.

The comparisons with the control group of the exclusion study as they stand indicate that with respect to the effects of limited surgical dissection, *and in the absence of exclusion*, the damage to the periosteum and soft tissue

after blunt trauma does not significantly affect the ability of these tissues to increase cortical flow. The *reduction* in marrow flow at one week in the present study supports the conclusion from the exclusion experiments that the early cortical flow response is mediated by a supply parallel to that to the marrow, probably that from the soft tissue. The mechanism of the changes in marrow flow are not clear but the recovery of flow by two weeks suggests that a temporary effect such as marrow oedema was responsible. This may have arisen from the division of the marrow by tearing rather than by the lesser trauma of an osteotomy.

The differences in skeletal muscle flow between the experimental and control limbs were more variable than in the exclusion experiments of Section 3.1, and in only one subgroup was statistical significance attained. This probably resulted from variation in the degree of trauma sustained by the muscle during the displacement of the fractures caused by blunt trauma, while muscle trauma during the surgical osteotomies was minimal, and varied less between different animals.

There were no significant differences between the higher and lower energy groups, and thus there is no evidence from this study that increasing the fracture energy significantly affects the blood flow to the healing fracture during the peak of the flow response. However, there are several reasons why such a difference may still exist.

Firstly, the statistical power of the study is low, with a maximum of six animals in any subgroup. The experimental to control ratios are, however, similar in the two groups (Table 3.2.3), particularly with regard to the cortex, and large numbers of animals would be required to demonstrate a statistical difference. For example, the equation of Lieber (1990) indicates that to establish statistical significance of the observed difference between the proximal cortices of the one week subgroups with 80% power and a significance level set at 5% would require over

two hundred animals. Even should such a result be achieved, the *clinical* significance of such a small difference would be doubtful.

Secondly, the difference between the two fracture energies may have been too small. The fractures in this series were not followed until union, and therefore there may have been no difference between the healing times of the two groups. The higher energy was set at a moderate level in order to avoid skin problems, but these appear to be minimal with the present method of three-point bending, in contrast to the crushing injuries of Edwards (1965b). Thus, further work with higher energy levels might demonstrate an effect on blood flow. It is difficult to relate the energies of this study with those of clinical injuries, as the latter are not known with any certainty and the rabbit tibia is much smaller than the human.

Thirdly, the fracture morphologies may have been inappropriate. Probably the most potent cause of osseous ischaemia at a fracture site is comminution, which is common in clinical high energy injuries. Apart from the small number of segmental fractures, comminution was minor in these experimental fractures, and should the principal mechanism of delayed union in higher energy injuries relate to this factor (Section 1.1.2), no difference between the present two groups would be expected. The formation of secondary fracture lines acts to dissipate quickly the applied energy, and the incidence of comminution increases with increasing rate of deformation (Sammarco *et al.* 1971). Thus, differences in flow might be found if the same energies are compared at higher velocities, with the mass of the weight reduced but its initial height increased (equations 3.1 and 3.3).

Fourthly, the blood flows measured in this experiment were necessarily average flows to the whole fragments. Thus, it is possible that in the higher energy group a larger or more deeply ischaemic area was created around the fracture site, associated with a greater reactive blood

flow response in the remainder of the diaphysis. This would result in little change in the overall average flow, while fracture site flow might be sufficiently reduced to retard healing. In view of similarity of the results from the two groups, this hypothesis requires that in both fragments the increased 'reactive' flow should match the local reduction in flow at the fracture site, which is unlikely. To investigate this further, a substantial increase in the number of microspheres injected would be required in order to enable smaller samples of the bone to be analysed accurately. A qualitative alternative would be to attempt autoradiography of the trapped microspheres in longitudinal sections, in order to map their distribution and to demonstrate the extent of any local ischaemic areas.

Fifthly, it is possible that the peak of the blood flow response after a fracture caused by blunt trauma occurs over a different period than that after osteotomy. In this case, measurements of flow before one week or later than two weeks might demonstrate a significant difference between the two energy groups. However, a difference lasting less than one week is unlikely to have a significant effect on healing, and any effect after two weeks would necessarily comprise a more rapid drop in flow towards normal in the higher energy group, and there is no reason to expect this.

Finally, the animal model may have been inappropriate. It is well known that delayed union is uncommon in small animals, and in view of the small distances involved in revascularisation of small bones it may be difficult to demonstrate differences in flow in the rabbit without the use of very severe violence.

Overall, the most probable causes for this experiment not demonstrating any real difference between the two groups are an inappropriately low higher energy level or the relative lack of fracture comminution. Future work might thus include increasing the mass of the weight or its velocity, in combination with adaptation of the support

mouldings in order to avoid the creation of segmental fractures. Changes in other parameters should also be considered. For example, the findings of Edwards (1965b) emphasise that the nature of the soft tissue injury has an important influence on tibial shaft healing when the energy of the fracture is kept virtually constant. Directing the impact onto the bone through muscle as well as skin would introduce another factor which may therefore differentiate the two energy groups.

In summary, the results of this study as they stand refute the hypothesis that higher energy tibial shaft fractures caused by blunt trauma are associated with a reduction in the blood flow response, at least during the peak period. This implies that delayed union in higher energy injuries is caused by factors other than an inadequate blood flow during healing. However, with alterations in the method, such as changes in the fracture morphology and in the energy levels utilised, and possibly a change of the experimental animal, such a hypothesis may yet receive experimental support.

Table 3.2.1 Lower energy group. Blood flows to the tibial diaphysis and the muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres one and two weeks after a unilateral lower energy tibial diaphyseal fracture. Flows are expressed as mean ± SE ml/min.100g (n=6 for each subgroup).

		Control		Fracture		p
proximal cortex	1	1.44 ± 0.47		2.10 ± 0.63		<0.02
	2	0.84	0.23	1.96	0.45	<0.05
distal cortex	1	2.55	0.90	2.98	0.97	NS
	2	1.77	0.51	3.88	0.98	<0.05
proximal marrow	1	23.91	8.18	12.40	4.64	<0.05
	2	24.16	5.38	20.72	5.44	NS
distal marrow	1	10.15	3.14	4.11	1.19	<0.05
	2	9.71	2.28	7.78	1.61	NS
skeletal muscle	1	2.89	0.63	8.50	2.80	NS
	2	2.82	0.51	9.34	4.22	NS

Control = control limb (no fracture)

Fracture = experimental limb

1 = one week subgroup

2 = two week subgroup

NS = not significant at 5% level (paired t test)

Table 3.2.2   Higher energy group.   Blood flows to the
tibial diaphysis and the muscle of the anterior
compartment, measured with 11.3 ± 0.1 (SD) µm microspheres
one and two weeks after a unilateral higher energy tibial
diaphyseal fracture. Flows are expressed as mean ± SE
ml/min.100g (n=5 for one week subgroup, n=6 for two week
subgroup).

		Control		Fracture		p
proximal cortex	1	0.39	± 0.07	0.69	± 0.18	NS
	2	0.61	0.10	1.58	0.47	NS
distal cortex	1	1.65	0.39	2.86	1.01	NS
	2	2.33	0.49	6.12	1.78	<0.05
proximal marrow	1	31.15	11.28	16.40	5.82	NS
	2	35.01	6.17	37.55*	5.77	NS
distal marrow	1	11.16	3.22	7.53	1.46	NS
	2	10.72	1.73	12.76	2.92	NS
skeletal muscle	1	2.68	0.43	3.43	0.96	NS
•	2	3.05	0.64	4.98	1.04	<0.05

Control = control limb (no fracture)

Fracture = experimental limb

1 = one week subgroup

2 = two week subgroup

NS = not significant at 5% level (paired t test)

* = significantly different from one week flow at 5% level
       (unpaired t test)

Table 3.2.3 Geometric means of the ratios between flows in
the experimental and control limbs   (n=6 for each subgroup
except n=5 for one week subgroup of higher energy group).

		Cast	Osteotomy	Lower	Higher
proximal cortex	1	0.81	2.05**	1.59**	1.66*
	2	0.95	3.17**	2.32**	2.41**
distal cortex	1	1.30	1.15	1.24	1.61*
	2	1.20	3.41**	2.23**	2.48**
proximal marrow	1	1.01	1.20	0.56*	0.28
	2	1.02	0.88	0.84	1.09
distal marrow	1	1.27	1.02	0.42**	0.70
	2	1.21	1.15	0.82	1.10
skeletal muscle	1	1.27	1.36	2.58	1.15
	2	1.17	2.14**	2.48	1.68**

Cast = cast immobilisation only (Section 2.5)

Osteotomy = osteotomy with cast (Section 3.1)

Lower = lower energy fracture with cast

Higher = higher energy fracture with cast

1 = one week subgroups

2 = two week subgroups

 * = significantly different from unity at 5% level (paired t test)

** = significantly different from unity at 1% level (paired t test)
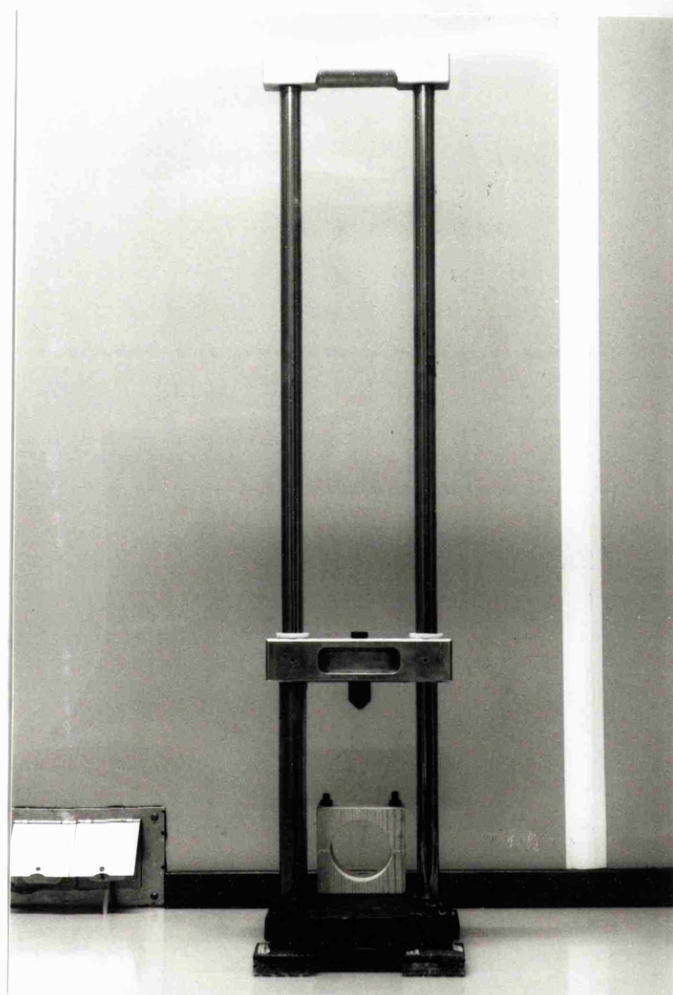
Figure 3.2.1 The fracture rig.

Note the wooden blocks, bolted to the baseplate, enclosing a circular opening for the hock mould.
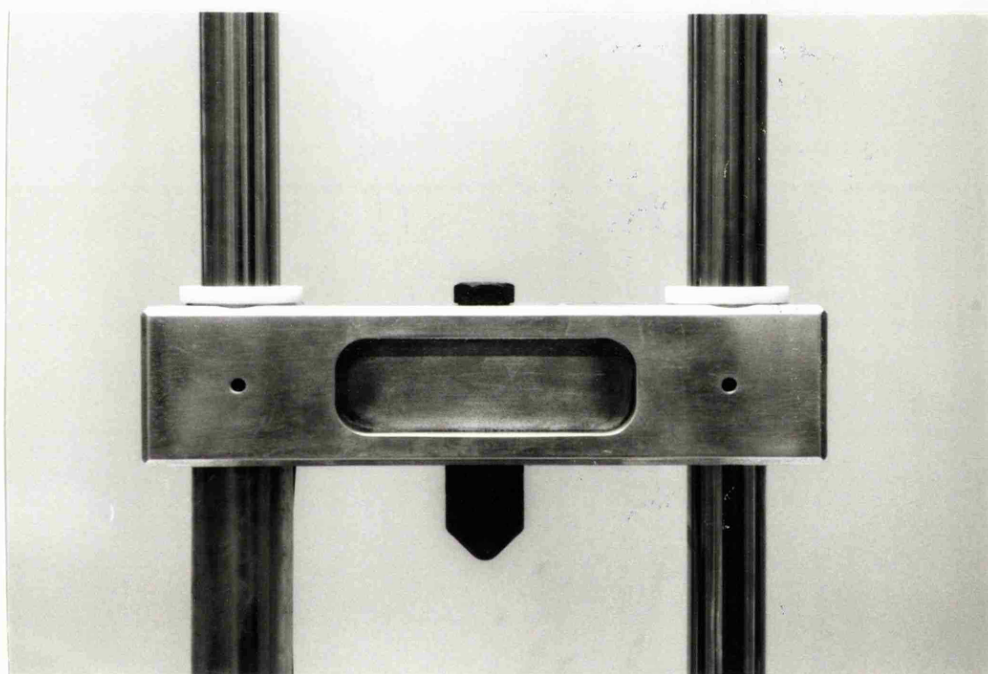
Figure 3.2.2   The rig weight.
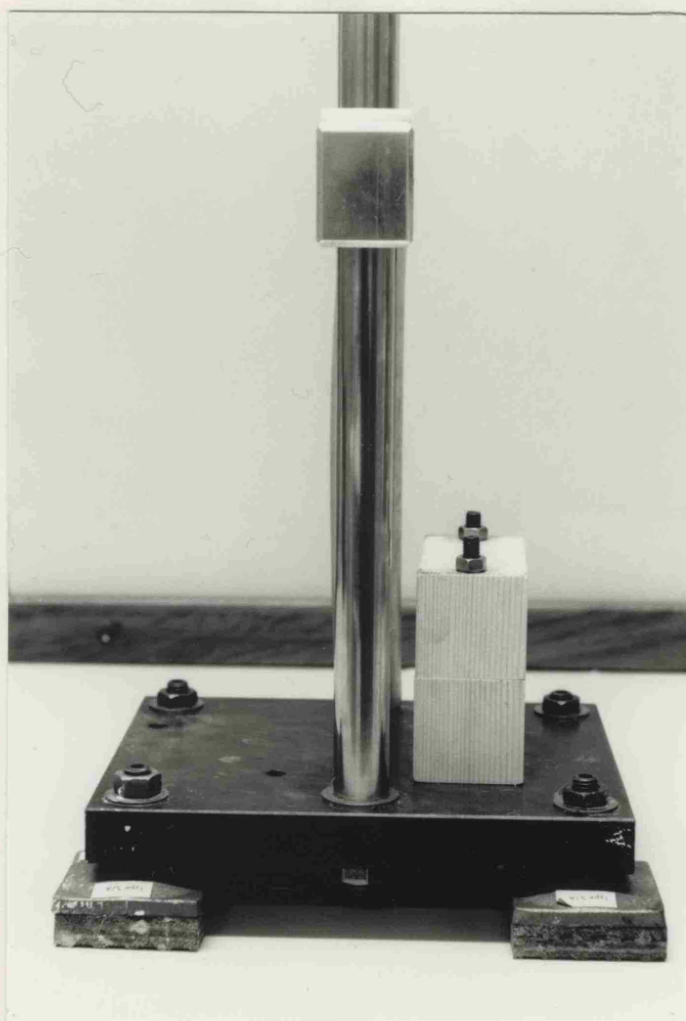
Figure 3.2.3   The rig base plate and mount for the hock
                   mould.

The wooden blocks to the right hold the hock mould. The knee mould is
held manually to the left of the vertical bars.

Figure 3.2.4   Radiograph of the tibial diaphysis after a transverse fracture produced by blunt trauma.

Figure 3.2.5   Radiograph of the tibial diaphysis after an oblique fracture produced by blunt trauma.

Figure 3.2.6 Radiograph of the tibial diaphysis after a segmental fracture produced by blunt trauma.

Figure 3.2.7

Lower energy group.
Radiographs of the tibial
diaphysis, one and two
weeks after transverse
fracture by blunt trauma.

(a) one week

(b) two weeks

Figure 3.2.8
Higher energy group.
Radiographs of the tibial
diaphysis, one and two
weeks after transverse
fracture by blunt trauma.

(a) one week

(b) two weeks

Figure 3.2.9   One week subgroups   Geometric means of the ratios between flows in the experimental and control limbs at one week   (n=6 for each subgroup except n=5 for higher energy group).

Geometric means of ratios
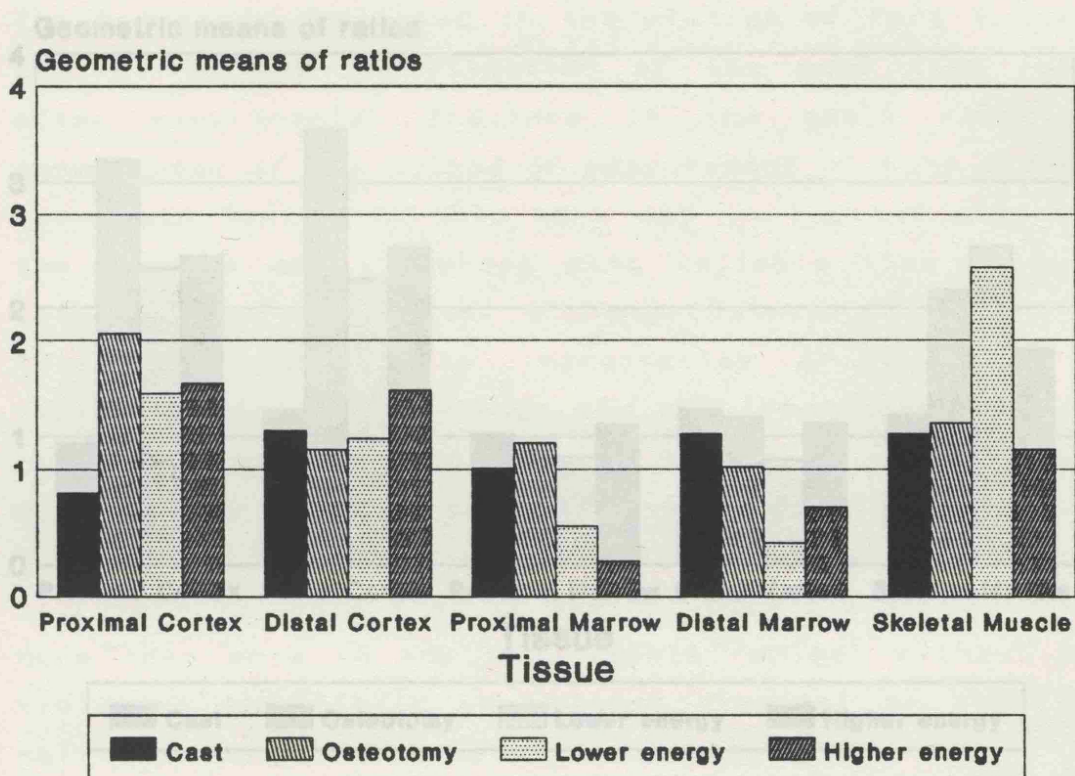
Figure 3.2.10 Two week subgroups Geometric means of the ratios between flows in the experimental and control limbs at two weeks (n=6 for each subgroup).
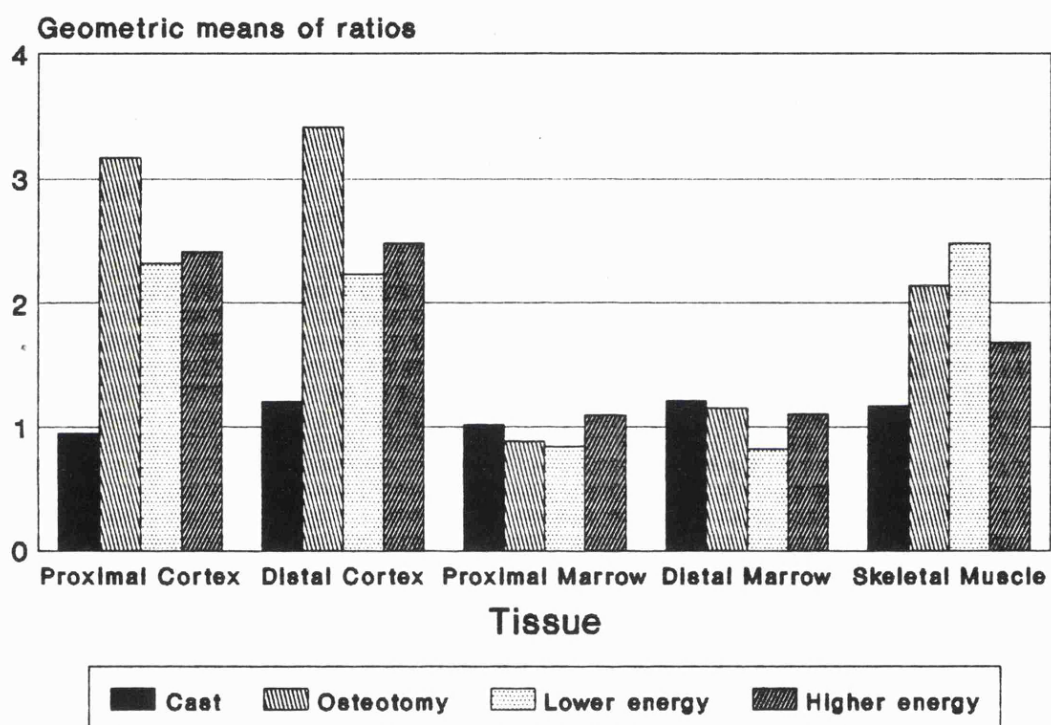
3.3

SUMMARY

The technique developed in the studies of Part 2 has been applied to the investigation of the peak flow response after experimental fracture in the adult rabbit. The assumptions of the method of measurement of bone blood flow have been tested in this work and in that of others, and the results are therefore more reliable than those from methods involving tracer exchange between blood and bone, which are subject to inaccuracies arising both from variations in such exchange and the interference caused by recirculation. The application of the microsphere method was limited in this part of the study after unannounced changes by the manufacturer in the specification of the microspheres, such that it was not possible to measure flow more than once in any experimental animal without either violating the limits on isotope disposal or reducing by half the number of animals investigated.

Almost all of the previous work in this field has considered flow to the fracture site as a whole, and has not distinguished flow to the marrow from flow to the cortex. Chidgey et al. (1986) used the microsphere method to measure flow in the cortex and marrow of one centimetre sections either side of a radial osteotomy in dogs, but the variation in results was very wide and no conclusions could be drawn. This variation between animals was not avoided in the present study, but more animals were investigated in each subgroup and differences between the bone proximal to an osteotomy of the tibia and that distal were demonstrated. Thus, while the flow to the proximal cortex was raised at one week, distally this was not observed until two weeks after osteotomy, presumably because of the

greater initial depression of cortical flow that occurs distally following division of the nutrient circulation (Kunze *et al.* 1981). Marrow flows were not found to differ between the control and experimental limbs at either time period. This indicates that the flow response in the cortex is mediated by a supply parallel to that of the marrow.

The investigation of the routes of supply after fracture by methods of tissue exclusion has yet to be extended to the use of a quantitative method, and flows have been measured in this study after exclusion of the periosteum and soft tissue or of the marrow by a sheath or intramedullary nail respectively. The cortical blood flow changes were abolished by the sheath, suggesting that the parallel supply to the cortex in the absence of exclusion is principally from the periosteum and soft tissues. Thus, despite the devascularisation of the cortex that is known to occur after medullary reaming, cortical flow was not significantly reduced by one or two weeks in the nailed group, and indeed was significantly raised distally by two weeks.

Such exclusion studies are open to criticism on the basis of the differences in osteotomy site mobility between the groups and the differences in the extent of exclusion along the tibial shaft. In this study, the short time periods obviated the probable principal effect of differences in mobility, that on the re-establishment of the medullary circulation across the osteotomy, and the sheath was found to be long enough effectively to exclude any rise in cortical flow arising from the soft tissues.

There has been no quantitative evidence put forward to support the concept of an increase in blood flow to the control limb after osteotomy, as suggested by perfusion studies. No statistically significant changes were found after simple osteotomy or after osteotomy with sheath exclusion, on comparison with the flows recorded after cast immobilisation alone (Section 2.5). However, at one week after a nailed osteotomy the flows to both cortex and

marrow of the control limb were higher than after simple immobilisation, but this change was found only in the distal marrow at two weeks. This indicated a general effect of the operative procedure, and this, together with the differences in this respect between the experimental groups, emphasises the importance of comparisons with the control limb in assessing the effects on flow arising solely from the experimental fracture, and raises doubts about the validity of studies of bilateral osteotomies treated by different methods.

The models used in the exclusion studies, while furnishing important information regarding the source of the peak flow response after experimental fracture, do not closely match the conditions following a clinical fracture. Thus, while the marrow circulation is interrupted and the soft tissue stripped from the bone by a fracture caused by blunt trauma, the circulations are able to re-establish without hindrance unless some form of internal fixation is used. No quantitative studies have been carried out on fractures caused by blunt trauma, and the hypothesis that higher energy injuries are associated with a reduced blood flow response has not been tested.

Fractures of the tibial shaft have been produced in the adult rabbit by percussion, with the bone supported at either end. This method proved successful in avoiding skin problems. By varying the mass of the weight used to produce the fractures it was possible to test the effect of increasing the fracturing energy. The blood flow changes after lower energy injuries, those in which the applied force was minimally above that required to produce a fracture, matched those found after a surgical osteotomy except that at one week both proximal and distal marrow flows were depressed. As the marrow flows were restored at two weeks, this change may have arisen from temporary oedema after the tearing of the marrow, which might not be expected to occur after the minimal marrow trauma of an osteotomy. The opposite directions of the changes in cortical and marrow flows confirmed the conclusion from the

exclusion studies that the increase in cortical flow after
fracture is mediated by a supply parallel to that to the
marrow.

Fractures produced by almost double the minimal
fracturing energy were also investigated. The data set at
one week was reduced by the exclusion of one animal with
skin breakdown, and therefore the changes in flow, which
were similar in nature to those of the lower energy group,
did not reach statistical significance on standard paired t
testing. At two weeks the flow to the distal cortex was
significantly raised, while that to the proximal cortex was
raised but not significantly so, and the marrow flows
matched those of the control limb. Thus the nature of the
changes in osseous tissue flow were similar in the two
energy groups, and the experimental to control limb ratios
were not found to differ between the groups for any tissue
at either time period.

Previous studies of bone blood flow after experimental
fracture have not included measurement of flow to the
neighbouring skeletal muscle. After osteotomy, involving
minimal muscle trauma, flow was found to increase on the
experimental side at two weeks, and this change was not
affected by the methods of exclusion. This change may have
reflected a general increase in soft tissue flow during the
mounting of the blood flow response to the osteotomy site.
After fractures produced by blunt trauma, in which muscle
trauma was probably more extensive, muscle flows were more
variable and this effect reached statistical significance
only in the higher energy group.

No evidence was found that higher energy fractures
produced by blunt trauma are associated with a depressed
blood flow response. Such an effect might be demonstrated
with the use of higher energy levels, or with a change in
method to produce more fracture comminution, which is
probably the most potent cause of bone ischaemia after
fracture. The results might also be different in a larger

animal where the distances involved in revascularisation are greater.

PART 4 —————————————————————————————

GENERAL DISCUSSION
AND SUMMARY

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4.1 ——————————————————————————————————

GENERAL DISCUSSION

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"Non-unions...have long been attributed to vascular factors without, however, any real knowledge of the basic circulatory changes"   Johnson (1927)

The tibia differs from other subcutaneous long bones such as the clavicle and ulna by the frequency with which it is fractured by direct violence of high energy. Such injuries are associated with comminution, severe displacement of the main fragments, and often loss of soft tissue cover, and these factors have been found in clinical studies to be related to delayed union. They might also be expected to be related to a high degree of devascularisation of the bone, which may constitute a potent cause of slowed healing.

The present study set out to answer two questions. Firstly, previous qualitative studies of tissue exclusion after experimental fracture had indicated that damage to the periosteal and soft tissue supply to the bone is of greater importance than marrow damage in the inhibition of union, but limited quantitative information was available as to the effect of such damage on the bone blood flow during healing. The question was posed as to what quantitative information can be obtained from the exclusion type of experiment concerning the relative rôles of the principal routes of blood supply to the tibial diaphysis in mounting the peak blood flow response after fracture. The clinical relevance of this question lies in the tailoring of the treatment of high energy fractures in order to maximise the blood flow available during healing. For example, such a study might indicate the degree of

importance of the restoration of vascular muscle cover after a compound fracture, and whether or not intramedullary nailing should be avoided.

In clinical terms, the routes of blood supply to the diaphysis are better defined by location rather than by vascular system, so that the external supply, from the periosteum and/or soft tissue, is contrasted with the internal supply within the marrow, rather than considering separately the muscular, periosteal, nutrient and epiphyseo-metaphyseal circulations. The importance of these external and internal supplies may be studied by exclusion of one or the other during healing.

While the blood flow to bone may be reduced by damage to its routes of supply, and this reduction may slow fracture healing if it persists, no experimental evidence was available that bone flow after higher energy fractures was attenuated in the absence of continued interference with the vascular systems. The second question posed was whether such experimental evidence could be given. High energy injuries would be expected to result in stripping of the periosteum and soft tissues from the bone during the displacement of the main fragments, and in the separation of fragments of bone altogether from their blood supply by comminution of the fracture site. Displacement of the main fragments will also cause disruption of the nutrient circulation. However, such damage might recover quickly in the absence of barriers such as experimental methods of exclusion or clinical methods of internal fixation.

To answer these questions, a reliable method is required to measure blood flow to the cortex, marrow and surrounding skeletal muscle. The radioactive microsphere method has major advantages over the other methods presently available (Section 1.2), and it is based on assumptions that can and should be tested. The investigation of the method for the measurement of blood flow in the tibia of the rabbit has been described in Part 2, and summarised in Section 2.6, while its

application to experimental fractures has been described in Part 3 and summarised in Section 3.3.

In answer to the first question, it was concluded from the exclusion studies that the soft tissues and/or periosteum were the principal source of the peak blood flow response after an osteotomy of the tibial shaft. It was not possible from these studies to consider the periosteum and surrounding muscle separately, as both were excluded or potentially damaged in the respective experiments, but the findings of perfusion studies suggest that the circulation of the muscle is the more prominent in the vascularisation of the cortex after fracture (Göthman 1960b,1961, Rhinelander 1974), although a collateral circulation exists between the two tissues (Zucman 1960, Whiteside and Lesker 1978a).

Previous qualitative investigations have suggested that separation, devascularisation or exclusion of these tissues from the fracture site results in delayed union (Sections 1.1.3 and 1.3.1), but a causative association between the inhibition of the flow response found in the exclusion experiment and such a delay in union is not certain. There was no attempt to establish such a link in the present study. No commonly used method of fracture treatment causes a similar circumferential separation of the diaphysis from the surrounding tissue, except cerclage wires or bands which do so only over short distances (Partridge 1976), although it has been suggested that the use of such devices is associated with delayed union (Jones 1986).

The observed increase in muscle flow by the second week after osteotomy would encourage the use of local flaps in the covering of soft tissue defects over the fractured tibia if bone blood flow is to be optimised. Richards and Schemitsch (1989), using the microsphere method in the dog, found that tibial diaphyseal segments removed and then replaced developed a higher cortical blood flow at thirty-one days if covered anteriorly by a local muscle flap rather than by simple closure of the skin. This difference

was not observed in the bone proximal and distal to the segment, but clinical union at this time was significantly more common in the muscle flap group. Fisher and Wood (1987) found that blood flow to free corticocancellous iliac grafts in the pig were higher when the grafts were placed under muscle rather than subcutaneously, suggesting that stimulation of the muscle flow by fracture is not a prerequisite for the beneficial effect of muscle cover, but revascularisation of bone by a free vascularised muscle flap taken from a remote site may not be as great as found with local muscle. It was notable in this connection that the flow to the muscle of the control limb in the present study did not increase after experimental fracture with respect to the flow observed after cast immobilisation alone, although the possibility remains that muscle flow responds to local humoral or mechanical factors in the fractured limb that would also act on any muscle transplanted to the area.

Destruction of the marrow circulation is commonly performed in the fixation of diaphyseal fractures by intramedullary nailing. In the present study, reaming and nailing did not prevent recovery of cortical flow to match that of the control limb by the end of the first week, and by the second week flow significantly exceeded the control value in the distal cortex. This implies that this technique of fracture management does not pose severe problems with regard to cortical blood flow, as appears to be the case clinically (Kessler et al. 1986). In clinical practice it is usual to ream the whole of the medullary tissue as well as the inner cortex, using powered instruments that result in fat embolisation of the Haversian canals (Danckwardt-Lillieström 1969, Danckwardt-Lillieström et al. 1970), and therefore the degree of initial cortical devascularisation is almost certainly greater than that caused in the present exclusion study. According to Kessler et al. (1986), such devascularisation does not affect comminuted fragments that have already lost their medullary blood supply, nor the formation of the

external callus that results in the healing of fractures treated by this method.

While it is important to study the changes in bone blood flow in the absence of bone fixation, future studies of tissue damage might now be carried out to confirm the present findings, using a form of bone fixation in order to reduce the variation in fracture site mobility between different groups. The least invasive method is external fixation, using pins inserted percutaneously at a distance from the fracture, and this has been successfully managed in the rabbit (Mawhinney *et al.* 1988, Brueton *et al.* 1990) and dog (Lewallen *et al.* 1984, Wu *et al.* 1984, Hart *et al.* 1985, Smith *et al.* 1987). The findings of the present exclusion study might be compared with the results of subperiosteal and extraperiosteal soft tissue stripping (Whiteside and Lesker 1978b) and of marrow reaming without nailing, thus creating fracture models with standardised tissue injury but without unphysiological exclusion. On the basis of the results of the exclusion study, recovery from all such procedures would be expected to be rapid in the rabbit, and a larger animal model would be preferable in order to match more closely the distances over which the revascularisation of bone must proceed in man.

In answer to the second question posed, no evidence was found in the study of fractures caused by blunt trauma to support the hypothesis that higher energy injuries are associated with a reduction in the bone blood flow response. This study indicated that recovery from any stripping of the periosteum and muscle from the bone was sufficient by the end of the first week for there to be no differences in cortical flow on comparison with the changes observed after a simple surgical osteotomy, this recovery occurring in the face of a reduced flow to the marrow. The reduction in marrow flow at one week after blunt trauma probably arose from local effects of trauma that would affect the supply to the cortex located in the marrow as

well as the supply to the marrow tissue itself, emphasising
the rôle of extra-osseous supply at this stage.

The rapidity with which extra-osseous tissues can mount
a blood flow response in isolated bone is demonstrated by
studies of diaphyseal grafts. The rabbit fibula, removed
and replaced in its bed, obtains a measurable blood flow
within five days, and flow exceeds that of the control limb
during the period between three and twenty weeks (Teissier
et al. 1985). In the dog, a free tibial diaphyseal graft
transplanted to the groin receives a measurable supply
within a week, and flow exceeds that of the control segment
in the unoperated limb by the end of the second week
(Siegert and Wood 1990). A similar rapidity of recovery
might be expected in bone stripped of its investing soft
tissues by trauma.

Thus there is no basis for special measures to preserve
or improve the bone blood flow after these fractures in an
attempt to reduce the incidence of delayed union. However,
an effect of fracture energy on subsequent bone blood flow
might have been found if the higher energy level tested had
been increased, or if the comparison had been carried out
using higher velocities so as to increase the degree of
fracture comminution.

The time periods of these studies were short, covering
the rise to the peak flow after fracture. Longer time
periods would have been required to allow an assessment of
any relationship between flow and time to union, although
objective measurement of the latter would be difficult
(Section 1.1.1). Mechanical testing in vitro is probably
the most accurate method of measurement of the stage of
healing, but this can be performed only once in each animal
at a predetermined time. Thus, unless large numbers of
animals are to be sacrificed, such a study would relate
flow to the degree of healing at a set time rather than to
the time taken to reach a set stage of healing. The latter
quantity has more clinical relevance. Testing in vivo

potentially overcomes this problem, but presents further difficulties both with technique and criteria.

Measurements of stiffness are meaningful only in the absence of internal fixation, and would necessitate the repeated removal and reapplication of any cast or external fixator used for immobilisation of the fracture, unless the frame of the fixator could itself be used to stress the bone. Accurate testing of the fracture site requires a firm interface between the testing apparatus and the bone, which is not possible in fractures held in a cast where there is no direct contact between the two, and the pins of external fixators are well known to loosen with time. Should pin loosening be avoided, testing can proceed only while the stiffness of the fracture is less than that of the pin-bone interface (Williams *et al.* 1987). Further, repeated anaesthesia will be required in order to give muscle relaxation, a requirement if the results are to be reproducible. This would also apply to assessment of union by vibration or ultrasound. In any event, the definition of 'union' from any of these tests is arbitrary, as the regaining of *normal* values takes longer than the time taken to reach *clinical* healing (Nokes *et al.* 1985, Williams *et al.* 1987).

Any difference in the degree of vascular damage between the two groups of fractures caused by blunt trauma appeared to be obviated by the rapidity of the recovery from that damage. Thus in the rabbit, longer experimental periods were not likely to have influenced the conclusions of the study. In a larger animal, longer periods may be necessary in view of the greater distances involved in revascularisation.

4.2

GENERAL SUMMARY

1. Union of fractures of the adult tibial shaft is often delayed, resulting in prolonged morbidity.

2. Clinical studies suggest that the fracture characteristics associated with delayed union are comminution, severe displacement, and compounding. These factors are commonly found in high energy injuries.

3. It is generally believed that a good blood supply is necessary for fracture healing. Comminution, displacement and soft tissue damage would be expected to result in devascularisation of the bone, and this may be the mechanism by which high energy injuries are associated with delayed union.

4. Delayed union is found in animal models where the blood supply is apparently reduced by surgical methods, although these methods have for the most part not been quantified. Union of free diaphyseal grafts appears more rapid if these are vascularised surgically via their nutrient vessels. However, in man it is not possible to measure bone blood flow non-invasively, and indirect methods have not given evidence in favour of the importance of ischaemia in delayed union.

5. The functional relationships of the cortical, periosteal and marrow blood vessels in the tibial diaphysis are not clearly defined at present. However, there does not appear to be the equivalent of a portal circulation, and this greatly simplifies the interpretation of measurements of bone blood flow.

6. The multiple arterial and venous vessels comprising the
    blood supply of the diaphysis prevent simple
    measurements of inflow and outflow, and tracer
    substances have been employed in order to measure bone
    blood flow indirectly. The measurement of clearance of
    tracers from blood by bone has major disadvantages
    arising principally from incomplete extraction of the
    tracer, and this method allows only one measurement in
    each animal. Marrow flow cannot be measured with the
    bone-seeking isotopes usually employed. Measurement of
    washout of tracer from bone by blood is subject to
    inaccuracies arising from possible diffusion limitation
    of exchange, and from recirculation. Furthermore, the
    tissue content of the tracers must be measured either
    locally by invasive means, or by external counting
    which is difficult to localise accurately.

7. The radioactive microsphere method overcomes these
    difficulties, and relies on simple assumptions that may
    be tested. It was therefore the method chosen for use
    in this study.

8. Measurements of bone blood flow taken using these
    various methods indicate that tibial cortical flow is
    very low, of the order of 1 to 5 ml/min.100g, while
    marrow flow varies between species. Approximately 70%
    of flow to the cortex appears to arise from the
    nutrient circulation, although the different methods of
    measurement give contradictory results.

9. Measurements of flows after experimental osteotomies of
    long bones indicate a peak in flow between seven and
    twenty one days. Flow is maximally increased near the
    osteotomy site, but rises are also seen in the
    remainder of the bone. Different forms of fixation have
    been found to be associated with differences in
    subsequent blood flow, with intramedullary nailing in
    general associated with the lowest flows. These
    studies, with single exceptions, have not distinguished
    flows proximal and distal to the osteotomy, and have

caused further interference with the bone by the use of internal or external fixation. The majority have relied on the uncertain methods of tracer clearance or washout, and no study has included fractures produced by blunt trauma.

10. Before the investigation of experimental fractures in the present study, a technique of measurement using radioactive microspheres in the adult rabbit was tested. Firstly, it was shown that two isotope labels could be reliably separated after suitable preparation of the samples. This included dissolution of the cortical samples in acid to overcome inaccuracies arising from their geometry.

11. Mixing of the microspheres at the site of injection was tested by comparing the flows to the tibial diaphyses of the two hindlimbs, which were found not to differ.

12. For optimum mixing of the microspheres in the major vessels, and for distribution of the microspheres within tissues to match as closely as possible that of erythrocytes, requires the use of microspheres close in size to erythrocytes while avoiding capillary non-entrapment. Soft tissue studies have indicated the optimum size to be 10μm or slightly above, but it is generally believed that microspheres nominally sized 15μm are the smallest that can be used in bone without significant capillary non-entrapment. The diffuse venous drainage of bone presents difficulties in the assessment of non-entrapment in this tissue, and this is most accurately investigated by comparison of calculated flows obtained with microspheres of two sizes injected simultaneously. It was found that microspheres sized 16.5 ± 0.1 (SD) μm and 11.3 ± 0.1 μm injected simultaneously gave calculated flows in tibial diaphyseal cortex and marrow, and in skeletal muscle, that were not significantly different. The calculated cortical flows were higher with the smaller spheres, possibly as a result of reduced steric restriction in

the small cortical vessels, but this effect was not
statistically significant. As smaller microspheres are
more evenly mixed in blood vessels, less likely to give
rise to central side-effects in large dosage, and
considerably cheaper, they were used for the remainder
of the study.

13. It has been found that a range of microsphere dosage of
up to 1.8×10^6 per kilogram body weight did not show
any tendency to reduce subsequent tibial diaphyseal and
skeletal muscle blood flow, and doses of microspheres
were kept within this range in the remaining
experiments.

14. Sequential measurements of blood flow were used to
quantify the early effects of cast immobilisation of
one hindlimb on tibial diaphyseal blood flow. A pilot
experiment indicated that leaching of isotope from the
microspheres *in vivo* was negligible. No differences
were found between the experimental and control limbs
after one or two weeks. This study demonstrated that
the opposite limb could be used as a control during the
investigation of blood flow to the tibia after a
unilateral experimental fracture immobilised in a cast,
allowing a reduction in the variation between animals
during statistical analysis.

15. The method of measurement of bone blood flow was
applied to unilateral surgical osteotomies of the
tibial diaphysis of the adult rabbit immobilised in a
cast. Flow to the proximal cortex was raised at one
week, while distally this was not observed until two
weeks after osteotomy, presumably because of the
greater initial depression of cortical flow that occurs
distally following division of the nutrient circulation
by an osteotomy. Marrow flows were not found to differ
between the control and experimental limbs at either
time period. This indicates that the flow response in
the cortex is mediated by a supply parallel to that to
the marrow.

16. Flows were also measured after exclusion of the periosteum and soft tissue or of the marrow by a sheath or intramedullary nail respectively. The cortical blood flow changes were abolished by the sheath, suggesting that the parallel supply to the cortex in the absence of exclusion is principally from the periosteum and soft tissues. Despite the devascularisation of the cortex that is known to occur after medullary reaming, cortical flow was not significantly reduced by one or two weeks, and indeed was significantly raised distally by two weeks. The short time period of the study obviated the likely principal effect of differences in the mobility of the osteotomy site between the groups, the variation in the inhibition of the re-establishment of the medullary circulation across the osteotomy.

17. No statistically significant changes were found in the flows to the tibial diaphysis of the control limb after simple osteotomy or after osteotomy with sheath exclusion, on comparison with the flows recorded after cast immobilisation alone. However, at one week after a nailed osteotomy the flows to both cortex and marrow of the control limb were higher than after simple immobilisation, but this change was found only in the distal marrow at two weeks. This indicated a general effect of the operative procedure, and this, together with the differences in this respect between the experimental groups, emphasises the importance of comparisons with the control limb in assessing the effects on flow arising solely from the experimental fracture. This finding also casts doubt on the validity of studies of bilateral osteotomies treated by different methods.

18. The hypothesis that higher energy injuries are associated with a reduced blood flow response was tested experimentally. Fractures of the tibial shaft were produced in the adult rabbit by percussion, with the bone supported at either end. This method proved successful in avoiding skin problems. By varying the

mass of the weight used to produce the fractures it was possible to test the effect of increasing the fracturing energy. The blood flow changes after lower energy injuries, those in which the applied force was minimally above that required to produce a fracture, were similar to those found after a surgical osteotomy in both proximal and distal cortex, while at one week both proximal and distal marrow flows were depressed. As the marrow flows were restored at two weeks, this change may have arisen from the tearing of the marrow causing temporary oedema, which might not be expected to occur after the minimal marrow trauma of an osteotomy. The opposite directions of the changes in cortical and marrow flows confirmed the conclusion from the exclusion studies that the increase in cortical flow after fracture is mediated by a supply parallel to that to the marrow.

19. Fractures produced by almost double the minimal fracturing energy were also investigated. The data set at one week was reduced by the exclusion of one animal with skin breakdown, and therefore the changes in flow, which were similar in nature to those of the lower energy group, did not reach statistical significance on standard paired t testing. At two weeks the flow to the distal cortex was significantly raised, while that to the proximal cortex was higher but not significantly so, and the marrow flows matched those of the control limb.

20. The nature of the changes in osseous tissue flow were similar in the two energy groups, and the experimental to control limb ratios were not found to differ between the groups for any tissue at either time period. No evidence was therefore found in this study that higher energy fractures produced by blunt trauma are associated with a depressed blood flow response. Such an effect might however be demonstrated with the use of yet higher energy levels, or with a change in method to produce more fracture comminution, which is probably

To be appended to paragraph 20

Thus, as the relationship of the energies used in this study to those encountered in clinical fractures are unknown, and the experimental fractures produced were not followed to union, a satisfactory "high" energy model associated with a degree of injury sufficient to result in delayed union and such as to test the effects of such trauma on the subsequent blood flow response may not have been achieved.

the most potent cause of bone ischaemia after fracture. The results might also be different in a larger animal where the distances involved in revascularisation are greater.

21. In the rabbit, the rapidity of the recovery of blood flow from the soft tissues after these fractures obviated any difference between them. This may also be true in man, as intramedullary nailing, which destroys the marrow circulation, may be used successfully in the management of high energy injuries. However, the soft tissue loss of severely compound injuries will prevent the re-establishment of extra-osseous supply, and vascular muscle transfers will be required if bone blood flow is to be maximised. There is experimental evidence from the study of diaphyseal grafts that this manœuvre hastens union. The rises in local muscle flow found in this study suggest that local flaps may be more effective in this regard than free flaps taken from a remote site. This type of injury has not been investigated in the present study, but the problems posed by the biomechanical testing of fractured bones *in vivo* makes the experimental demonstration of a relationship between reduced bone blood flow and delayed union in such a model difficult.

APPENDICES

APPENDIX 1 ────────────────────────────────

SPECTRAL STRIPPING AND GAMMA COUNTER CALIBRATION

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SEPARATION OF EMISSION SPECTRA

The measurement of gamma radiation depends upon the interactions of the incipient gamma rays with the electrons of the detector medium (Knoll 1989). The energies of the emissions of Gd^{153} and Sn^{113} are at a level where two types of interaction predominate: photoelectric absorption and Compton scattering.

In *photoelectric absorption* essentially all the energy of the gamma photon is transferred to an electron in a bound shell of an absorber atom, and this results in disappearance of the photon. With *Compton scattering*, the photon transfers only part of its energy to an electron, and continues along its deflected path. The resultant recoil electron thus ranges in energy from very little up to nearly the energy of the incident gamma photon.

The electrons produced by these interactions are detected, and their energy measured, by the scintillation they produce in the detector medium. This light energy is in turn measured by photomultipliers, whose output is usually plotted as a *differential pulse height spectrum* (Fig. A.1). The abscissa of this spectrum is the electron energy (in practice, the voltage of the pulse produced by the photomultipliers), and the ordinate is the differential with respect to the electron energy of the number of pulses of that energy per unit time. The number of pulses recorded within a range of energies is therefore represented by the area under the curve over the width of that range.

The spectrum comprises two principal parts, the *Compton continuum* and the *photopeak*. With mono-energetic emissions, the energy of the photopeak corresponds to the energy of the incident gamma photons released by photoelectric absorption. In detectors of a practical size, the gap between the highest energy arising from single Compton scattering and that of the photopeak is bridged by the summation of multiple Compton scattering of a proportion of the photons.

The separation of the emissions from two isotopes contained in a single sample is complicated by the Compton continuum of the more energetic isotope overlapping the spectrum of the less energetic. However, under constant conditions, the ratio of the counts in the continuum to those in the photopeak is constant, and this can be used to separate the isotopes by the technique of *spectral stripping* (Heymann *et al*. 1977).

The isotopes are selected such that their photopeaks are sufficiently different in energy to be measured separately by the counter. The energy window may then be set to allow the recording only of counts in the photopeak of the more energetic isotope. The count is then repeated with the window set to the band containing the photopeak of the less energetic isotope, together with the overlying part of the Compton continuum of the first isotope. If the counter has more than one channel, these counts can be obtained simultaneously. As the number of counts in the first photopeak is known, and the ratio of the continuum to the photopeak can be determined by calibration experiments, the continuum count can be calculated and then subtracted from the second count to give the counts arising solely from the second isotope (Fig. A.2). Thus :

$$count_1 = channel_1 \times (1 + k) \qquad\qquad (A.1)$$

$$count_2 = channel_2 - (k \times channel_1) \qquad\qquad (A.2)$$

where $count_1$ = counts arising from higher energy isotope

$count_2$ = counts arising from lower energy isotope

$channel_1$ = counts measured in higher energy window

$channel_2$ = counts measured in lower energy window

k = ratio of counts of the higher energy isotope in channel 2 to counts in channel 1

After setting the windows of the two channels of the counter to maximise the counts from each isotope while excluding any counts from the lower energy isotope Gd^{153} from channel 1, the counter was calibrated to determine both the value of k for Sn^{113}, and the effects of sample height on counter efficiency.

COUNTER CALIBRATION

The gamma counter used in this study was a type 80000, manufactured by LKB Instruments Ltd, Selsdon.   .

The determination of k

The standard method of determination of the degree of spillover from channel 1 to channel 2 of the higher energy isotope is simply to count samples of the isotope over a range of activities, and to obtain an average proportion or ratio (Heymann *et al.* 1977). However, this method did not give accurate resolution of mixtures of the two isotopes. A technique to measure k in mixed samples was therefore developed.

Eight pairs of microsphere aliquots, one of each pair labelled with Gd^{153} and the other with Sn^{113}, were prepared and counted separately. In each pair, the activities of the two isotopes in channel 2 were approximately equal, and these activities ranged from approximately 2000 counts per minute (cpm) to 11000 cpm. All samples were centrifuged to ensure constant geometry of the microspheres in relation to the detector (see below). In four cases the Sn^{113} aliquot was added to its Gd^{153} counterpart, and in the other four

the reverse. As not all the microspheres could be
transferred from one tube to another, the remainder were
recounted after recentrifugation, to allow calculation of
the activity actually transferred. The mixtures were also
centrifuged and counted, and the value of k required to
recover the original Gd[153] count determined. Thus, from
equation A.2 :

$$k = (channel_2 - count_2) / channel_1 \qquad (A.3)$$

where $count_2$, that of the Gd[153], is known from the counts
obtained before mixing (Table A.1).

Linear regression equations were calculated from the
results, and expressions for k in terms of the channel 1
and channel 2 counts thereby obtained. It was found that k
was more closely related to the channel 2 count, with a
correlation coefficient close to 0.9, and $p < 0.01$. This
equation was that used in the computer program for the
calculation of blood flows.

Calibration for varying specimen height

The efficiency of a gamma radiation counter varies with the
geometry of the sample and its distance from the detector
(Katz and Blantz 1972, Mernagh et al. 1976, Heymann et al.
1977, Knoll 1989). The geometry and distance of the
microspheres in the reference blood samples of the
microsphere method may be made constant by centrifugation,
and the changes in efficiency with increasing height of the
tissue samples related to this configuration. This
calibration was carried out using a modification of the
technique of Heymann et al. (1977).

Aliquots of 25μl of stock suspensions of the
microspheres labelled with Gd[153] or Sn[113] were placed at
the bottom of counting tubes, ten for each isotope. A
20% (w/v) solution of gelatin at approximately 60°C was

poured into nine of the tubes to depths varying up to
4·5cm. A few drops of the solution were placed in the tenth
tube, and this tube placed in a cold room at 3 ± 1°C for
the gelatin to set with the microspheres held on the floor
of the tubes. This tube represented 'zero' height.

The other tubes were placed on a vortex mixer for
approximately 30 seconds to suspend the microspheres
throughout the height of the gelatin column. They were then
placed in the cold room for rapid setting of the gelatin,
holding the microspheres in their suspended positions. The
actual heights of the columns were measured with a ruler to
the nearest 0·5mm.

Counts for both channels were obtained over 1 minute
for each tube, accumulating at least 10^5 counts for each
isotope. All tubes, except those with zero height, were
then placed in a water bath at approximately 60°C for six
hours to remelt the gelatin and allow the microspheres to
settle to the zero height position. The water bath was
turned off and the gelatin allowed to reset. The tubes were
recounted as before.

The efficiency of counting for each height was
expressed as the ratio between the suspended microsphere
count and the settled microsphere count, after correcting
for background activity and the delay between the two
counts.

The ratios for each isotope were divided by that for
the zero height tube (giving a zero height ratio of 1) to
correct for any variation between the two counts arising
from factors other than the change in microsphere
configuration. The normalised ratios were then subjected to
linear regression analysis. The ratios and results of the
analysis are given in Table A.2. The high p value obtained
for Gd^{153} at the lower heights resulted from there being
only four data points; the degree of correlation to a
linear fit remains high.

   These equations were used in the computer program
developed to calculate blood flows, in order to correct the
tissue counts to the reference sample configuration.

COUNT CORRECTION FOR DELAYS

The decay of isotopic activity is exponential, having the
form :

$$activity_t = initial\ activity \times e^{-kt} \qquad (A.4)$$

where $activity_t$ = activity at time t.

The half-life ($t_{\frac{1}{2}}$), *ie* the time taken for the initial
activity to drop to half its original value, is known for
each isotope :

$$\tfrac{1}{2} \times initial\ activity =$$
$$initial\ activity \times e^{-kt_{\frac{1}{2}}} \qquad (A.5)$$

and therefore :

$$k = log_e\ 2\ /\ t_{\frac{1}{2}} \qquad (A.6)$$

These were the equations used in the computer program
developed to calculate blood flows (Appendix 2), to correct
for any delay between the counting of the reference blood
samples and the tissue samples.

MEASUREMENT OF MICROSPHERE NUMBERS

The importance of the actual number of microspheres in each
sample lies in the assessment of the likely effect on

calculated blood flows of random variation in microsphere distribution (Katz *et al.* 1981, Buckberg *et al.* 1971, Li *et al.* 1989). This is discussed in Section 2.2.

The method used in this study was that of Warren and Ledingham (1974). A drop of a dilute suspension of the microspheres was spread over a 1 × 1cm piece of graph paper and allowed to dry. The paper was taped to a microscope slide and the spheres counted using a magnification of ×100. The paper was then placed at the bottom of a counting vial and the radioactivity counted. The specific activity in counts per microsphere was obtained by dividing the total counts by the number of microspheres. As the half-life of each isotope was known, the specific activity could be calculated for any subsequent day.

For each specimen, the number of microspheres labelled by each isotope could be obtained by dividing the specimen count for that isotope by the corrected specific activity.

Table A.1.   Counts per minute of microsphere aliquots
labelled with Gd[153] and Sn[113], separate and mixed, with
calculated values of the channel 2 to channel 1 ratio for
tin (k) in the mixtures.

Pair	Gd[153]	Sn[113]		Mixture		
no.	ch2	ch2	ch1	ch2	ch1	k
1	1930	3129	6054	4817	5453	0.5294
2	4789	4932	9444	9411	7987	0.6012
3	5570	6045	11462	10773	9075	0.5733
4	7750	8345	15558	14986	12952	0.5862
5	8710	9465	18657	17404	13978	0.6220
6	9901	9834	19055	19003	15775	0.6141
7	11553	11980	23393	22348	16815	0.6420
8	12710	10743	21501	23272	15267	0.7106

ch1 = counts per minute channel 1

ch2 = counts per minute channel 2

Linear regression analysis of the relationship of k to the
mixture counts:

$$k = 0.4908 + 9.820 \times 10^{-6}.\text{ch1}$$

correlation coefficient = 0.7647, $p < 0.03$

$$k = 0.4997 + 7.249 \times 10^{-6}.\text{ch2}$$

correlation coefficient = 0.8866, $p < 0.01$

Table A.2. Normalised ratios of counts obtained at varying heights to counts at zero height.

Gd153		Sn113		
Ht.	ch2	Ht.	ch2	ch1
0	1.0000	0	1.0000	1.0000
1.0	1.0042	1.25	0.9367	0.9139
1.7	1.0272	1.3	0.9376	0.9108
2.25	1.0491	1.65	0.9779	0.9104
2.75	1.0341	2.45	0.9249	0.8948
3.3	1.0336	2.5	0.8776	0.8589
3.85	1.0102	2.95	0.9168	0.8762
4.2	1.0016	3.75	0.8682	0.8624
4.5	1.0095	3.85	0.8904	0.8486
		4.4	0.8535	0.8350

ch2 = channel 2 window

ch1 = channel 1 window

Ht. = height of gelatin column in centimetres

Linear regression analysis of the relationship of each ratio to specimen height:

For Gd-153 at heights less than 2.39cm:
$$ratio = 0.9933 + 0.02168.height$$
correlation coefficient = 0.9265, $p < 0.1$

For Gd-153 at heights greater than 2.39cm:
$$ratio = 1.0930 - 0.02015.height$$
correlation coefficient = 0.9434, $p < 0.006$

For Sn-113, channel 2:
$$ratio = 0.9909 - 0.03009.height$$
correlation coefficient = 0.8625, $p < 0.002$

For Sn-113, channel 1:
$$ratio = 0.9715 - 0.03336.height$$
correlation coefficient = 0.9200, $p < 0.001$

Figure A.1   The differential pulse height spectrum   (after
             Knoll 1989).
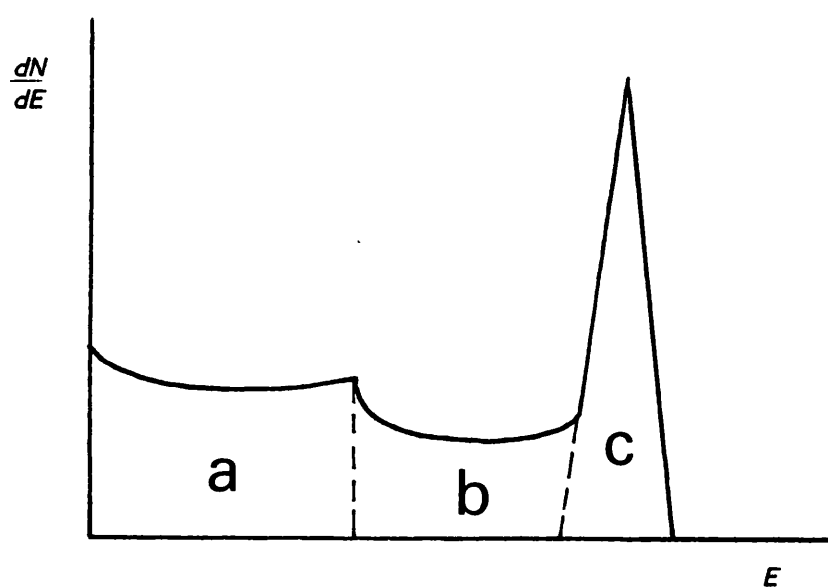
N = number of pulses per unit time

E = energy of emission

a = single Compton scattering

b = multiple Compton scattering

a + b = Compton continuum

c = photopeak

Figure A.2 Spectral stripping of the Compton continuum of a more energetic isotope from the photopeak of a lesser energetic isotope.

$$\frac{dN}{dE}$$

channel 1   channel 2

a

b

c

E
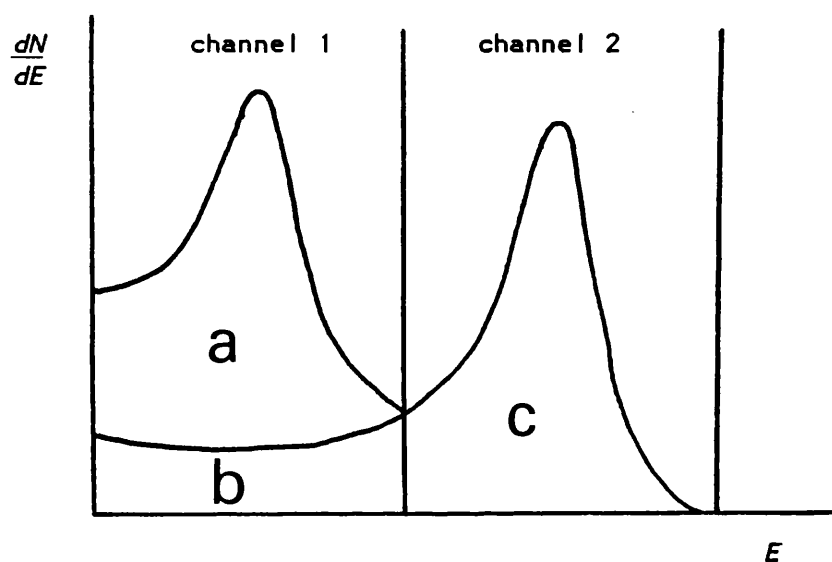
a = photopeak of less energetic isotope

b = Compton continuum of more energetic isotope

c = photopeak of more energetic isotope

ratio k = b / c

APPENDIX 2 ——————————————————————————————

COMPUTER PROGRAMS

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PROGRAMS DEVELOPED DURING THE STUDY

Twenty one programs were written by the author during the course of the study, ranging from short routines of a few lines to the main programs for entering and processing the experimental data. Programming was in *Mallard BASIC* (Locomotive Software Ltd, Dorking), run on a *PCW8512* microcomputer (Amstrad Consumer Electronics plc, Brentwood). A list of programs is given below, and the listings for the main programs are given on the following pages.

Short programs

DECAY : generation of Gd^{153} and Sn^{113} decay tables.

EXFRACT : calculation of percentage excretion of isotope from microspheres *in vivo* (Section 2.5).

GDHEIGHT : calculation of changes in Gd^{153} counts with increasing height of sample (Appendix 1).

KMIX : calculation of *k* by mixture (Appendix 1).

LEACH% : calculation of leaching of isotopes into acid supernatant (Section 2.2).

RECALC2 : generation from the results files of tables of the number of specimens with greater than 150 microspheres (Parts 2 and 3).

RECALC4 : generation of ratios of sequential flows and microsphere dosages from results files, for regression analysis (Section 2.4).

SNHEIGHT : calculation of changes in Sn^{113} counts with increasing height of sample (Appendix 1).

SPACTDEL : generation of tables of days elapsed, for calculation of corrected microsphere specific activity (Appendix 1).

Statistics programs

Ten programs were written for the retrieval and direct analysis of the blood flow results.

Main programs

DATENTRY : interactive program for the entry, checking and filing onto disc of experimental data

FLOW   : program for the calculation of blood flows from the files of experimental data, incorporating spectral stripping and corrections for specimen height, and filing of results onto disc for subsequent statistical analysis. This program took three forms, for the calculation of simultaneous, sequential, and single flow measurements. The form for simultaneous flows is given below.

MAIN PROGRAM LISTINGS

Datentry

```
10 default=10000
20 prog$="flow"
30 markX=-1
40 :
50 REM Screen Handling
60 e$=CHR$(27)
70 cls$=CHR$(27)+"E"+CHR$(27)+"H"
80 inverse$=CHR$(27)+"p"
```

```
90 normal$=e$+"q"

100 cursoff$=e$+"f"

110 curson$=e$+"e"

120 DEF FNc$(x%,y%)=e$+"Y"+CHR$(32+y%)+CHR$(32+x%)

130 DEF FNw$(x1%,y1%,x2%,y2%)=e$+"X"+CHR$(32+y1%)+CHR$(32+x1%)+
    CHR$(32+y2%-y1%-1)+CHR$(32+x2%-x1%-1)

140 ww$=FNw$(0,0,90,30)

150 ww2$=FNw$(0,3,90,28)

160 PRINT ww$;cls$:ok=FRE("")

170 PRINT inverse$;"DATENTRY";normal$

180 :

190 PRINT FNw$(30,5,60,25):PRINT cursoff$

200 PRINT "N  new data file":PRINT

210 PRINT "R  retrieve data":PRINT

220 PRINT "F  file data to disc":PRINT

230 PRINT "C  change/add data":PRINT

240 PRINT "L  ";prog$:PRINT:PRINT

250 PRINT "X  exit"

260 y$=INKEY$:IF y$="" THEN 260

270 IF y$="n" THEN PRINT curson$:GOTO 370

280 IF y$="r" THEN GOTO 1750

290 IF y$="f" THEN GOTO 1390

300 IF y$="c" THEN GOTO 1870

310 IF y$="l" THEN PRINT ww$;cls$;"Loading":CHAIN prog$

320 IF y$="x" THEN PRINT ww$:PRINT cls$:PRINT "Program end":
    PRINT curson$:END

330 GOTO 260

340 :

350 REM *** NEW DATA FILE ***

360 :

370 PRINT ww2$:PRINT cls$

380 INPUT "New filename (M: drive)";data$:PRINT:PRINT

390 IF INSTR(data$,"m:")=0 AND INSTR(data$,"M:")=0 THEN GOTO 380

400 IF FIND$(data$)<>"" THEN PRINT "Filename already in use":
    PRINT:PRINT:GOTO 380

410 OPEN "O",1,data$

420 INPUT "Title of data";mtitle$:PRINT

430 INPUT "Number of experiments";n%:PRINT
```

```
440 WRITE #1,mtitle$,nX
450 PRINT "Present channel 2 default count is";default;SPACE$(15);
    "change ?  [y/n]"
460 y$=INKEY$:IF y$="" THEN 460
470 IF y$="n" THEN GOTO 500
480 IF y$="y" THEN PRINT:INPUT "New default";default:GOTO 500
490 GOTO 460
500 xmX=1
510 xX=xmX
520 PRINT cls$
530 PRINT "EXPERIMENT";xX:PRINT FNw$(0,6,90,28)
540 IF xX<(xmX+1) THEN GOTO 600
550 IF xX>xmX THEN PRINT "Repeat ref sample number and backgrounds ?
    [y/n]":PRINT
560 y$=INKEY$:IF y$="" THEN GOTO 560
570 IF y$="y" THEN WRITE #1,refnoX,bg1,bg2:GOTO 640
580 IF y$="n" THEN GOTO 600
590 GOTO 560
600 INPUT "Number of reference samples";refnoX:PRINT
610 WRITE #1,refnoX
620 INPUT "Background for reference samples (channels 1 and 2)";
    bg1,bg2
630 WRITE #1,bg1,bg2
640 PRINT cls$
650 PRINT "Gadolinium reference samples - input time":PRINT
660 PRINT TAB(15);"time":PRINT
670 FOR y=1 TO refnoX
680 PRINT y;TAB(15);
690 INPUT time
700 IF time=0 THEN PRINT CHR$(7):GOTO 680
710 count=5.4E+07/time
720 WRITE #1,count
730 NEXT y
740 PRINT cls$
750 PRINT "Tin reference samples - input time and channel 1 counts":
    PRINT
760 PRINT TAB(15);"time";TAB(30);"ch1 count":PRINT
770 FOR y=1 TO refnoX
```

```
780 PRINT y;TAB(15);
790 INPUT;time:PRINT TAB(30);:INPUT count
800 count1=count*600/time:count2=default*600/time
810 WRITE #1,count1,count2
820 NEXT y
830 PRINT cls$
840 :
850 PRINT TAB(5);"delay";TAB(15);"Gd spact";TAB(30);"Sn spact";
860 PRINT TAB(45);"Gd spact delay";TAB(65);"Sn spact delay":PRINT
870 IF x%>xm% THEN PRINT FNc$(30,20);"? repeat [y/n]";:GOSUB 1630
880 PRINT TAB(5);:INPUT;delay
890 PRINT TAB(15);:INPUT;Gdspact
900 PRINT TAB(30);:INPUT;Snspact
910 PRINT TAB(45);:INPUT;Gdspactdel
920 PRINT TAB(65);:INPUT;Snspactdel
930 WRITE #1,delay,Gdspact,Snspact,Gdspactdel,Snspactdel
940 PRINT cls$
950 :
960 PRINT "Same background for bone samples ? [y/n]":PRINT
970 y$=INKEY$:IF y$="" THEN 970
980 IF y$="y" THEN WRITE #1,bg1,bg2,default,default,default,default,
    default,default:GOTO 1050
990 IF y$="n" THEN GOTO 1010
1000 GOTO 970
1010 PRINT TAB(25);"background":PRINT
1020 PRINT "Channel 1";TAB(25);:INPUT bbg1:PRINT
1030 PRINT "Channel 2";TAB(25);:INPUT bbg2
1040 WRITE #1,bbg1,bbg2,default,default,default,default,default,
     default
1050 PRINT cls$
1060 PRINT TAB(15);"weight";TAB(30);"height";TAB(45);"time";TAB(60);
     "ch1 count":PRINT
1070 PRINT "EU";:GOSUB 1270
1080 PRINT "CU";:GOSUB 1270
1090 PRINT "EL";:GOSUB 1270
1100 PRINT "CL";:GOSUB 1270
1110 PRINT CHR$(7);"continue ?  [y/n]"
1120 y$=INKEY$:IF y$="" THEN 1120
```

```
1130 IF y$="y" THEN GOTO 1160
1140 IF y$="n" THEN item=1:FOR i%=1 TO 24:WRITE #1,item:NEXT:GOTO 1220
1150 GOTO 1120
1160 PRINT "EM";:GOSUB 1270
1170 PRINT "CM";:GOSUB 1270
1180 PRINT "EUM";:GOSUB 1270
1190 PRINT "CUM";:GOSUB 1270
1200 PRINT "ELM";:GOSUB 1270
1210 PRINT "CLM";:GOSUB 1270
1220 WRITE #1,mark%:PRINT ww2$
1230 IF x%=n% THEN CLOSE 1:GOTO 1390
1240 x%=x%+1:GOTO 520
1250 CLOSE 1:GOTO 1390
1260 :
1270 PRINT TAB(15);:INPUT;weight
1280 IF weight>2.5 OR weight<0.3 THEN PRINT CHR$(7);:tb%=15:NW=weight:
     GOSUB 1560:weight=NW
1290 PRINT TAB(30);:INPUT;height
1300 IF height>3.5 THEN PRINT CHR$(7);:tb%=30:NW=height:GOSUB 1560:
     height=NW
1310 PRINT TAB(45);:INPUT;time
1320 IF time>20000 OR time<1000 THEN PRINT CHR$(7);:tb%=45:NW=time:
     GOSUB 1560:time=NW
1330 PRINT TAB(60);:INPUT count
1340 IF count>80000! OR count<3000 THEN PRINT CHR$(7);:tb%=60:
     NW=count:GOSUB 1560:count=NW
1350 count1=count*600/time:count2=default*600/time
1360 WRITE #1,weight,height,count1,count2
1370 PRINT:RETURN
1380 :
1390 PRINT ww2$:PRINT cls$:PRINT cursoff$
1400 IF data$="" THEN PRINT "No data":FOR q=1 TO 2000:NEXT:GOTO 160
1410 PRINT "Saving to disc - press any key when disc available in B:
     drive":PRINT
1420 y$=INKEY$:IF y$="" THEN 1420
1430 PRINT "Present filename is ";data$
1440 PRINT:INPUT "New filename (B:drive)";bdata$
```

```
1450 IF INSTR(data$,"m:") <> 0 AND INSTR(bdata$,"m:") <> 0
     THEN PRINT:PRINT "Destination file should be on B: drive":
     GOTO 1440
1460 IF FIND$(bdata$)<>"" AND data$<>bdata$ THEN GOTO 2400
1470 OPEN "I",1,data$:OPEN "O",2,bdata$
1480 INPUT #1,mtitle$,n%:WRITE #2,mtitle$,n%
1490 WHILE NOT EOF(1)
1500 INPUT #1,item
1510 WRITE #2,item
1520 WEND
1530 CLOSE
1540 PRINT:PRINT "  Done":FOR q=1 TO 1000:NEXT:GOTO 160
1550 :
1560 PRINT TAB(tb%+10);"check [y/n]":PRINT TAB(tb%)
1570 y$=INKEY$:IF y$="" THEN 1570
1580 IF y$="y" AND tb%<60 THEN RETURN
1590 IF y$="y" AND tb%=60 THEN PRINT:RETURN
1600 IF y$="n" THEN PRINT TAB(tb%);:INPUT;NW:RETURN
1610 GOTO 1570
1620 :
1630 y$=INKEY$:IF y$="" THEN 1630
1640 IF y$="y" THEN GOTO 930
1650 IF y$="n" THEN PRINT FNc$(0,2):RETURN
1660 GOTO 1630
1670 :
1680 :
1690 y$=INKEY$:IF y$="" THEN 1690
1700 IF y$="x" THEN CLOSE:GOTO 160
1710 PRINT ww2$;cls$:INPUT #1,mark%:IF x=n THEN CLOSE:GOTO 160
1720 NEXT x
1730 CLOSE:GOTO 160
1740 :
1750 PRINT ww2$;cls$:PRINT:PRINT "Directory of B drive disc:":PRINT:
     PRINT
1760 DIR b:*.dat
1770 PRINT:PRINT:PRINT
1780 PRINT "Directory of M drive:":PRINT:PRINT
1790 IF FIND$("m:*.dat")="" THEN PRINT "no files":GOTO 1810
```

```
1800 DIR m:*.dat
1810 PRINT:PRINT:PRINT:PRINT
1820 INPUT "Name of file (including drive)";data$
1830 IF data$="x" THEN GOTO 160
1840 IF FIND$(data$)="" THEN PRINT:PRINT "File not found":
     PRINT:GOTO 1820
1850 GOTO 160
1860 :
1870 REM ** CHANGE DATA **
1880 :
1890 IF data$="" THEN GOTO 1750
1900 PRINT cls$;"C  change data point":PRINT
1910 PRINT "A  add data":PRINT
1920 PRINT "S  save new file to disc":PRINT:PRINT "X  exit"
1930 y$=INKEY$:IF y$="" THEN 1930
1940 IF y$="x" THEN GOTO 160
1950 IF y$="s" THEN data$=ndata$:GOTO 1390
1960 IF y$="c" THEN GOTO 1990
1970 IF y$="a" THEN GOTO 2560
1980 GOTO 1930
1990 PRINT ww2$;cls$
2000 INPUT "Name of new file (including drive)";ndata$:PRINT
2010 IF ndata$="x" THEN GOTO 2380
2020 INPUT "Experiment number (0 to exit)";number%:PRINT
2030 IF number%=0 THEN GOTO 160
2040 PRINT:PRINT "  Loading file"
2050 OPEN "I",1,data$:OPEN "O",2,ndata$:INPUT #1,mtitle$,n:
     WRITE #2,mtitle$,n
2060 q%=1
2070 WHILE q%<number%
2080 INPUT #1,item:WRITE #2,item
2090 IF item<0 THEN q%=q%+1
2100 WEND
2110 z%=1
2120 PRINT:PRINT "Change?  [y/n]"
2130 INPUT #1,item
2140 IF item=-1 AND EOF(1)=0 THEN GOTO 2240
     ELSE IF item=-1 AND number%=n THEN PRINT:PRINT:
```

```
          PRINT "End of file":WRITE #2,item:CLOSE:data$=ndata$:
          FOR q=1 TO 2000:NEXT:PRINT cls$;FNw$(30,5,60,25):
          GOTO 1900
2150 PRINT FNc$(5,10);z%;item;SPACE$(10)
2160 y$=INKEY$:IF y$="" THEN 2160
2170 IF y$="n" THEN WRITE #2,item:z%=z%+1:GOTO 2130
2180 IF y$="y" THEN GOTO 2210
2190 IF y$="x" THEN WRITE #2,item:GOTO 2330
2200 GOTO 2160
2210 PRINT:INPUT "New value";newv
2220 WRITE #2,newv:z%=z%+1:PRINT cls$:GOTO 2120
2230 :
2240 WRITE #2,item
2250 PRINT FNc$(5,15);"End of data":PRINT:PRINT
2260 PRINT "E   next experiment":PRINT
2270 PRINT "X   exit"
2280 y$=INKEY$:IF y$="" THEN 2280
2290 IF y$="e" THEN PRINT cls$:number%=number%+1:GOTO 2110
2300 IF y$="x" THEN PRINT cls$;"  Sorting files":GOTO 2330
2310 GOTO 2280
2320 :
2330 WHILE NOT EOF(1)
2340 INPUT #1,item:WRITE #2,item
2350 WEND
2360 PRINT:PRINT "  Done":FOR q=1 TO 2000:NEXT:CLOSE
2370 data$=ndata$
2380 PRINT cls$;FNw$(30,5,60,25):GOTO 1900
2390 :
2400 PRINT:PRINT "File already exists":PRINT
2410 PRINT "  Press C  to overwrite":PRINT
2420 PRINT "         A  to abandon"
2430 y$=INKEY$:IF y$="" THEN 2430
2440 IF y$="c" THEN KILL bdata$:GOTO 1470
2450 IF y$="a" THEN GOTO 1440
2460 GOTO 2430
2470 :
2480 IF ndata$<>data$ THEN CLOSE:KILL ndata$:PRINT:GOTO 2020
2490 WRITE #2,item
```

```
2500 PRINT:PRINT "  Sorting files":PRINT
2510 WHILE NOT EOF(1)
2520 INPUT #1,item:WRITE #2,item
2530 WEND
2540 CLOSE:GOTO 2020
2550 :
2560 PRINT ww2$;cls$
2570 INPUT "Name of new file (including drive)";adata$:PRINT
2580 IF adata$="x" THEN GOTO 2380
2590 OPEN "I",2,data$:OPEN "O",1,adata$
2600 INPUT #2,mtitle$,n%
2610 PRINT "Present number of experiments is";n%:PRINT
2620 INPUT "Number of new experiments";newn%:PRINT
2630 xm%=n%+1:n%=n%+newn%:WRITE #1,mtitle$,n%
2640 PRINT "  creating new file ";
2650 WHILE NOT EOF(2)
2660 INPUT #2,item:WRITE #1,item
2670 IF item<0 THEN PRINT ". ";
2680 WEND
2690 CLOSE 2:data$=adata$:GOTO 510
2700 :
```

Flow

```
10 ok=FRE("")
20 cls$=CHR$(27)+"E"+CHR$(27)+"H"
30 cursoff$=e$+"f"
40 curson$=e$+"e"
50 DEF FNc$(x%,y%)=e$+"Y"+CHR$(32+y%)+CHR$(32+x%)
60 DEF FNw$(x1%,y1%,x2%,y2%)=e$+"X"+CHR$(32+y1%)+CHR$(32+x1%)+
   CHR$(32+y2%-y1%-1)+CHR$(32+x2%-x1%-1)
70 ww$=FNw$(0,0,90,30)
80 PRINT cursoff$;cls$
90 GOTO 200
100 :
110 REM *INKEYS SUB*
120 y$=INKEY$
130 WHILE y$=""
```

```
140 y$=INKEY$
150 WEND
160 RETURN
170 :
180 REM ** SET-UP **
190 :
200 klowGd=0.02168:khighGd=0.02015:kSn1=0.03009:kSn2=0.03336:j=2.4
210 ylow=0.99329:yhigh=1.09303:ySn1=0.99089:ySn2=0.9715
220 sn1=0.29929:sn2=0.70071:refflow=2.6755:d1=12:d=10
230 DEF FNdgd(x)=EXP(-0.002864*x)
240 DEF FNdsn(x)=EXP(-0.006022*x)
250 DIM weight(d1,d):DIM height(d1,d):DIM count(d1,2,d):
    DIM Sncount(d1,d):DIM Gdcount(d1,d)
260 DIM Snflow(d1,d):DIM Gdflow(d1,d)
270 DIM Snno(d1,d):DIM Gdno(d1,d)
280 GOTO 1150
290 :
300 INPUT "Name of data file";data$:PRINT:PRINT
310 IF FIND$(data$)="" THEN PRINT:PRINT "File not found":
    PRINT:GOTO 300
320 PRINT "Press any key when ready":PRINT cursoff$
330 GOSUB 120
340 PRINT cls$:PRINT FNc$(30,5);"CALCULATING   "
350 :
360 REM ** READ **
370 :
380 OPEN "I",1,data$
390 INPUT #1,mtitle$,number%,n%,refflow
400 FOR x%=1 TO number%
410 INPUT #1,refno,bg1,bg2
420 ref1=0:ref2=0
430 FOR y=1 TO refno
440 INPUT #1,count
450 NEXT y
460 FOR y=1 TO refno
470 INPUT #1,count1,count2
480 kref=0.49966+7.249E-06*count1
490 ref1=ref1+count1-bg1-kref*(count2-bg2):
```

```
        ref2=ref2+count2-bg2+kref*(count2-bg2)
500 NEXT y
510 refGd=ref1:refSn=ref2
520 PRINT FNc$(43,5);x%
530 INPUT #1,delay,spactGd,spactSn,spactGddel,spactSndel
540 INPUT #1,bg1,bg2,item,item,item,item,item,item
550 FOR specimen%=1 TO n%
560 INPUT #1,weight(x%,specimen%),height(x%,specimen%)
570 INPUT #1,count(x%,1,specimen%),count(x%,2,specimen%)
580 count(x%,1,specimen%)=count(x%,1,specimen%)-bg1
590 count(x%,2,specimen%)=count(x%,2,specimen%)-bg2
600 NEXT:INPUT #1,mark%:IF mark%>0 THEN PRINT CHR$(7);"read error"
610 GOSUB 660
620 NEXT x%:CLOSE 1:title$="FULL DATA":GOTO 1750
630 :
640 REM ** CALCULATE **
650 :
660 spactSndel=FNdsn(spactSndel):spactSn=spactSn*spactSndel
670 spactGddel=FNdgd(spactGddel):spactGd=spactGd*spactGddel
680 IF delay<1 THEN delaySn=1 ELSE GOTO 700
690 IF delay<1 THEN delayGd=1:GOTO 710
700 delaySn=FNdsn(delay):delayGd=FNdgd(delay)
710 FOR specimen%=1 TO n%
720 k=0.49966+7.249E-06*count(x%,1,specimen%):kh=k
730 Sncount(x%,specimen%)=count(x%,2,specimen%)
740 Gdcount(x%,specimen%)=
        count(x%,1,specimen%)-(kh*Sncount(x%,specimen%))
750 Sncount(x%,specimen%)=Sncount(x%,specimen%)/delaySn
760 Gdcount(x%,specimen%)=Gdcount(x%,specimen%)/delayGd
770 IF height(x%,specimen%)=0 THEN GOTO 820
780 Sncount(x%,specimen%)=
        Sncount(x%,specimen%)/(ySn2-kSn2*height(x%,specimen%))
790 IF height(x%,specimen%)<j THEN GOTO 800 ELSE GOTO 810
800 Gdcount(x%,specimen%)= Gdcount(x%,specimen%)/
        (ylow+klowGd*height(x%,specimen%)):GOTO 820
810 Gdcount(x%,specimen%)=Gdcount(x%,specimen%)/
        (yhigh-khighGd*height(x%,specimen%))
820 Sncount(x%,specimen%)=Sncount(x%,specimen%)*(1+k)
```

```
830 Snflow(xX,specimenX)=100*refflow*Sncount(xX,specimenX)/
    (refSn*weight(xX,specimenX))
840 Gdflow(xX,specimenX)=100*refflow*Gdcount(xX,specimenX)/
    (refGd*weight(xX,specimenX))
850 Snno(xX,specimenX)=Sncount(xX,specimenX)*delaySn/
    spactSn
860 Gdno(xX,specimenX)=Gdcount(xX,specimenX)*delayGd/
    spactGd
870 IF specimenX>10 THEN RETURN
880 NEXT specimenX
890 RETURN
900 :
910 REM ** DISPLAY **
920 :
930 PRINT cls$
940 IF data$="" THEN PRINT "NO DATA":FOR q=1 TO 2000:NEXT:GOTO 1150
950 FOR x=1 TO numberX
960 PRINT mtitle$;" - ";title$:PRINT
970 PRINT x;TAB(10);"Gd flow";TAB(25);"Sn flow";TAB(40);"Gd no.";
    TAB(55);"Sn no.":PRINT
980 FOR y=1 TO nX
990 Snflow(x,y)=ROUND(Snflow(x,y),2)
1000 Gdflow(x,y)=ROUND(Gdflow(x,y),2)
1010 Snno(x,y)=ROUND(Snno(x,y))
1020 Gdno(x,y)=ROUND(Gdno(x,y))
1030 PRINT TAB(4);y;TAB(10);Gdflow(x,y);TAB(25);Snflow(x,y);TAB(40);
    Gdno(x,y);TAB(55);Snno(x,y)
1040 NEXT:PRINT:PRINT:PRINT:PRINT
1050 PRINT "press RETURN to continue"
1060 GOSUB 120
1070 IF y$="x" THEN RETURN
1080 PRINT cls$
1090 NEXT
1100 RETURN
1110 ok=FRE("")
1120 :
1130 :
1140 ok=FRE(""):PRINT curson$:PRINT "program end":PRINT:END
```

```
1150 ok=FRE(""):PRINT cls$:PRINT FNw$(30,5,80,80)

1160 PRINT "D  for display":PRINT

1170 PRINT "P  for printing":PRINT

1180 PRINT "F  to file results":PRINT

1190 PRINT "B  to retrieve results":PRINT

1200 PRINT "N  for new data":PRINT

1210 PRINT "T  for stats program":PRINT:PRINT

1220 PRINT "X  for exit":PRINT:PRINT:PRINT cursoff$

1230 GOSUB 120:PRINT ww$:PRINT cls$

1240 match$="dpfbntx":aX=INSTR(match$,y$)

1250 IF aX=5 THEN PRINT cls$:GOTO 300

1260 ON aX GOSUB 930,1330,1500,1790,1260,1290,1140

1270 GOTO 1150

1280 :

1290 PRINT cls$;"Loading STATS":CHAIN "stats"

1300 :

1310 REM ** PRINT **

1320 :

1330 IF data$="" THEN PRINT "NO DATA":FOR q=1 TO 2000:NEXT:GOTO 1150

1340 PRINT cls$:PRINT FNc$(35,5);"PRINTING":PRINT FNc$(35,25);title$

1350 LPRINT "FLOWS FOR ";data$;"    ";mtitle$;" - ";title$;
     "  ( n =";numberX;")    (GGH)":LPRINT:LPRINT

1360 FOR x=1 TO numberX

1370 LPRINT x;TAB(10);"Gd flow";TAB(25);"Sn flow";TAB(40);"Gd no.";
     TAB(55);"Sn no.":LPRINT

1380 FOR y=1 TO nX

1390 Snflow(x,y)=ROUND(Snflow(x,y),2)

1400 Gdflow(x,y)=ROUND(Gdflow(x,y),2)

1410 Snno(x,y)=ROUND(Snno(x,y))

1420 Gdno(x,y)=ROUND(Gdno(x,y))

1430 LPRINT TAB(4);y;TAB(10);Gdflow(x,y);TAB(25);Snflow(x,y);TAB(40);
     Gdno(x,y);TAB(55);Snno(x,y)

1440 NEXT:LPRINT:LPRINT

1450 NEXT

1460 RETURN

1470 :

1480 REM ** FILE RESULTS **

1490 :
```

```
1500 IF data$="" THEN PRINT "NO DATA":FOR q=1 TO 2000:NEXT:GOTO 1150
1510 PRINT ww$;cls$;"FILE RESULTS":PRINT:PRINT
1520 PRINT title$:PRINT:PRINT
1530 PRINT "Present filename is ";data$:PRINT
1540 INPUT "Name of file";bdata$
1550 IF bdata$="x" THEN GOTO 1150
1560 IF bdata$=data$ THEN PRINT:GOTO 1540
1570 IF FIND$(bdata$)<>"" THEN GOTO 1690
1580 IF INSTR(bdata$,".res")=0 THEN PRINT:PRINT " file must be .res
     file":PRINT:GOTO 1540
1590 PRINT:PRINT:PRINT " filing ";
1600 OPEN "O",1,bdata$
1610 WRITE #1,mtitle$,title$,number%,n%
1620 FOR x%=1 TO number%
1630 FOR y%=1 TO n%
1640 WRITE #1,Gdflow(x%,y%),Snflow(x%,y%),Gdno(x%,y%),Snno(x%,y%)
1650 NEXT y%:PRINT ". ";:NEXT x%
1660 CLOSE 1:PRINT:PRINT:PRINT " Done":
1670 FOR q=1 TO 2000:NEXT:GOTO 1150
1680 :
1690 PRINT:PRINT "File already exists":PRINT
1700 PRINT " Press C to overwrite":PRINT
1710 PRINT "         A to abandon":PRINT
1720 GOSUB 120
1730 IF y$="c" THEN GOTO 1590
1740 IF y$="a" THEN PRINT:GOTO 1540
1750 GOTO 1720
1760 :
1770 REM ** RETRIEVE RESULTS **
1780 :
1790 PRINT "RETRIEVE RESULTS":PRINT:PRINT
1800 PRINT "Directory of B drive":PRINT:PRINT
1810 DIR b:*.res
1820 PRINT:PRINT:PRINT
1830 PRINT "Directory of M drive":PRINT:PRINT
1840 IF FIND$("m:*.res")="" THEN PRINT "No files":GOTO 1860
1850 DIR m:*.res
1860 PRINT:PRINT:PRINT:INPUT "Name of file (including drive)";data$
```

```
1870 IF data$="x" THEN GOTO 1150
1880 IF FIND$(data$)="" THEN PRINT:GOTO 1860
1890 PRINT:PRINT " loading ";
1900 OPEN "I",1,data$
1910 INPUT #1,mtitle$,title$,numberX,nX
1920 FOR xX=1 TO numberX
1930 FOR yX=1 TO nX
1940 INPUT #1,Gdflow(xX,yX),Snflow(xX,yX),Gdno(xX,yX),Snno(xX,yX)
1950 NEXT yX:PRINT ". ";:NEXT xX
1960 CLOSE 1:PRINT:PRINT:PRINT " Done":FOR q=1 TO 2000:NEXT:GOTO 1150
1970 :
```

APPENDIX 3 ————————————————————

EXPERIMENTAL RESULTS

Below are given the results of the blood flow measurements described in Parts 2 and 3. Use is made of the following abbreviations :

No.	:	identification number of experimental animal
Ms number	:	number of microspheres in each sample
R	:	right hindlimb
L	:	left hindlimb
E	:	experimental limb (cast alone, osteotomy with cast, or fracture with cast)
C	:	control limb

Table A.3.1   Section 2.2   Blood flows to the tibial diaphysis and muscle of the anterior compartment, measured simultaneously with 11.3 ± 0.1 (SD) μm microspheres labelled with Gd[153] and Sn[113]. The dimension of flow is ml/min.100g. Cortical samples counted intact.

No.			Flow Gd[153]	Flow Sn[113]	Ms number Gd[153]	Ms number Sn[113]
1	proximal cortex	R	0.62	0.84	76	94
		L	0.77	0.73	88	77
	distal cortex	R	1.15	1.45	116	134
		L	2.01	1.75	184	146
	proximal marrow	R	30.37	36.53	1306	1432
		L	38.99	35.53	1582	1314
	distal marrow	R	8.49	6.18	207	137
		L	10.60	7.29	258	162
	skeletal muscle	R	6.40	5.25	717	536
		L	8.72	9.61	1103	1108
2	proximal cortex	R	0.61	0.84	46	82
		L	1.04	1.01	81	104
	distal cortex	R	0.50	1.02	34	91
		L	0.71	0.70	49	63
	proximal marrow	R	59.43	49.03	1407	1522
		L	47.94	42.57	1189	1385
	distal marrow	R	23.45	16.30	436	398
		L	12.56	13.65	255	363
	skeletal muscle	R	14.08	12.96	1142	1380
		L	14.38	10.48	891	852

Table A.3.1 (continued)

No.			Flow		Ms number	
			Gd153	Sn113	Gd153	Sn113
3	proximal cortex	R	2.02	2.32	234	297
		L	1.97	2.70	229	345
	distal cortex	R	1.70	2.41	177	276
		L	1.92	2.75	205	324
	proximal marrow	R	33.52	36.21	725	862
		L	29.07	33.35	934	1180
	distal marrow	R	4.70	5.69	102	135
		L	12.52	7.65	249	167
	skeletal muscle	R	22.57	19.82	2624	2537
		L	15.06	15.51	1574	1786
4	proximal cortex	R	2.81	4.71	192	452
		L	2.96	5.58	203	536
	distal cortex	R	2.63	3.55	150	284
		L	2.34	3.95	137	323
	proximal marrow	R	39.39	40.13	545	778
		L	31.84	37.60	451	747
	distal marrow	R	25.34	17.12	280	266
		L	19.22	19.62	173	247
	skeletal muscle	R	16.73	15.30	1001	1283
		L	13.46	12.73	861	1141
5	proximal cortex	R	1.91	2.52	173	228
		L	1.86	1.98	173	185
	distal cortex	R	2.05	2.77	176	237
		L	1.71	2.23	145	188
	proximal marrow	R	69.38	70.06	1251	1261
		L	83.14	88.94	1766	1887
	distal marrow	R	17.36	14.79	235	200
		L	15.49	15.13	239	233
	skeletal muscle	R	21.25	20.07	2298	2168
		L	24.78	26.77	2697	2909

Table A.3.1 (continued)

No.			Flow		Ms number	
			Gd153	Sn113	Gd153	Sn113
6	proximal cortex	R	3.57	4.07	406	629
		L	2.67	3.58	338	615
	distal cortex	R	5.88	5.81	591	793
		L	5.00	5.62	481	735
	proximal marrow	R	26.96	25.92	746	974
		L	10.75	8.30	931	977
	distal marrow	R	9.43	10.93	199	313
		L	8.02	9.83	174	289
	skeletal muscle	R	2.45	2.10	253	295
		L	1.89	2.10	233	352
7	proximal cortex	R	4.31	6.20	401	442
		L	2.67	4.31	236	292
	distal cortex	R	2.89	4.48	221	263
		L	2.48	3.55	186	205
	proximal marrow	R	74.98	83.64	1545	1323
		L	78.45	79.91	1545	1208
	distal marrow	R	10.82	15.16	164	176
		L	15.34	16.00	232	186
	skeletal muscle	R	24.09	35.18	1192	1336
		L	17.64	16.03	784	547
8	proximal cortex	R	1.19	1.22	141	137
		L	1.80	1.90	231	230
	distal cortex	R	2.29	2.47	236	239
		L	2.64	2.95	282	297
	proximal marrow	R	27.83	31.58	831	890
		L	23.09	25.30	650	672
	distal marrow	R	14.01	12.85	306	265
		L	15.20	14.06	332	290
	skeletal muscle	R	10.56	10.69	685	655
		L	8.62	6.61	441	319

Table A.3.1 (continued)

No.			Flow		Ms number	
			Gd¹⁵³	Sn¹¹³	Gd¹⁵³	Sn¹¹³
9	proximal cortex	R	1.57	2.18	103	201
		L	2.08	2.32	141	221
	distal cortex	R	1.34	1.75	77	140
		L	1.93	2.64	109	210
	proximal marrow	R	6.97	7.13	137	198
		L	5.14	9.78	95	255
	distal marrow	R	3.07	1.69	53	41
		L	2.18	2.62	39	67
	skeletal muscle	R	7.07	10.72	184	392
		L	12.21	12.94	293	438
10	proximal cortex	R	2.15	3.09	94	237
		L	1.82	2.75	84	223
	distal cortex	R	1.96	4.03	79	283
		L	2.40	4.13	89	266
	proximal marrow	R	14.78	20.54	248	602
		L	16.24	20.71	277	616
	distal marrow	R	6.74	7.39	96	183
		L	4.35	6.07	63	153
	skeletal muscle	R	7.50	8.31	163	315
		L	7.20	7.12	173	299
11	proximal cortex	R	7.03	9.39	409	223
		L	8.60	10.06	483	230
	distal cortex	R	11.89	15.93	516	281
		L	11.96	16.77	591	338
	proximal marrow	R	58.39	63.82	649	288
		L	56.06	57.60	585	245
	distal marrow	R	35.42	37.90	346	151
		L	28.78	31.67	300	134
	skeletal muscle	R	9.64	11.15	321	151
		L	10.42	12.35	452	218

Table A.3.1 (continued)

No.			Flow		Ms number	
			Gd153	Sn113	Gd153	Sn113
12	proximal cortex	R	3.38	5.04	230	130
		L	3.41	3.63	241	97
	distal cortex	R	4.20	5.12	233	108
		L	4.56	6.31	256	135
	proximal marrow	R	40.01	47.84	537	243
		L	33.58	45.42	440	226
	distal marrow	R	5.92	4.91	62	20
		L	7.64	7.86	85	33
	skeletal muscle	R	4.59	7.14	144	85
		L	4.28	5.75	129	65

Table A.3.2 Section 2.2 Blood flows to the cortex of the tibial diaphysis, measured simultaneously with 11.3 ± 0.1 (SD) µm microspheres labelled with Gd153 and Sn113, counted after dissolution in 5.8 N hydrochloric acid. The dimension of flow is ml/min.100g.

No.			Flow		Ms number	
			Gd153	Sn113	Gd153	Sn113
1	proximal cortex	R	0.44	0.70	54	78
		L	0.69	0.74	79	77
	distal cortex	R	1.12	1.39	114	129
		L	specimen lost in centrifuge			
2	proximal cortex	R	0.55	0.84	41	82
		L	0.93	0.89	72	91
	distal cortex	R	0.51	1.04	35	93
		L	0.71	0.65	48	58
3	proximal cortex	R	1.96	2.13	228	273
		L	2.06	2.57	239	329
	distal cortex	R	1.86	2.08	193	238
		L	2.08	2.38	223	281
4	proximal cortex	R	3.10	3.76	212	361
		L	3.23	4.33	221	415
	distal cortex	R	2.83	3.31	162	265
		L	2.48	3.80	145	311
5	proximal cortex	R	1.89	2.95	174	277
		L	1.50	2.40	142	232
	distal cortex	R	2.12	3.06	184	271
		L	1.75	2.65	150	232
6	proximal cortex	R	3.74	4.25	432	680
		L	2.76	3.76	354	669
	distal cortex	R	6.06	5.71	619	808
		L	5.13	5.56	502	753

Table A.3.3   Section 2.2 Blood flows to the cortex of the tibial diaphysis, measured simultaneously with 11,3 ± 0.1 (SD) µm microspheres labelled with Gd153 and Sn113, counted after dissolution in 11.6 N hydrochloric acid. The dimension of flow is ml/min.100g.

No.			Flow Gd153	Flow Sn113	Ms number Gd153	Ms number Sn113
7	proximal cortex	R	2.17	2.36	235	186
		L	1.68	1.96	173	147
	distal cortex	R	1.71	2.14	152	138
		L	1.53	1.69	133	108
8	proximal cortex	R	0.47	0.31	61	43
		L	0.50	0.68	70	99
	distal cortex	R	1.40	1.61	157	189
		L	1.77	1.94	205	236
9	proximal cortex	R	0.79	1.40	61	178
		L	1.02	1.34	81	177
	distal cortex	R	0.77	0.96	52	107
		L	1.17	1.36	78	149
10	proximal cortex	R	0.89	1.55	46	137
		L	0.74	1.13	41	105
	distal cortex	R	1.16	2.53	55	205
		L	1.67	2.46	73	182
11	proximal cortex	R	3.45	4.02	207	99
		L	4.90	4.73	284	112
	distal cortex	R	10.26	11.05	458	203
		L	8.95	11.46	456	240
12	proximal cortex	R	2.74	3.26	193	86
		L	2.40	2.37	176	65
	distal cortex	R	1.20	1.21	69	26
		L	1.23	1.80	72	39

Table A.3.4   Section 2.3   Blood flows to the tibial diaphysis and muscle of the anterior compartment, measured simultaneously with 16.5 ± 0.1 (SD) µm and 11.3 ± 0.1 µm microspheres. The dimension of flow is ml/min.100g.

No.			Flow 16.5µm	Flow 11.3µm	Ms number 16.5µm	Ms number 11.3µm
1	proximal cortex	R	1.12	1.60	42	152
		L	1.56	1.72	59	163
	distal cortex	R	1.37	1.59	46	133
		L	2.76	2.35	87	186
	proximal marrow	R	46.73	41.42	379	844
		L	56.48	47.38	507	1069
	distal marrow	R	19.14	19.87	125	327
		L	16.25	17.36	98	263
	skeletal muscle	R	20.43	19.04	493	1156
		L	43.90	39.09	727	1628
2	proximal cortex	R	1.13	1.28	47	161
		L	1.32	1.43	54	174
	distal cortex	R	1.72	2.13	63	233
		L	1.67	1.98	58	207
	proximal marrow	R	40.95	38.91	382	1086
		L	33.61	35.45	286	903
	distal marrow	R	15.66	19.33	108	398
		L	12.66	26.83	81	512
	skeletal muscle	R	10.01	9.57	201	576
		L	7.82	6.55	183	459

Table A.3.4 (continued)

No.			Flow		Ms number	
			16.5μm	11.3μm	16.5μm	11.3μm
3	proximal cortex	R	5.09	6.85	192	586
		L	4.19	6.10	168	555
	distal cortex	R	6.23	7.90	209	602
		L	5.83	6.40	191	476
	proximal marrow	R	21.27	18.93	148	299
		L	18.43	20.18	132	328
	distal marrow	R	6.39	7.07	33	83
		L	9.56	9.63	51	117
	skeletal muscle	R	13.26	15.63	293	782
		L	16.69	19.09	255	663
4	proximal cortex	R	1.61	2.13	27	134
		L	1.50	2.40	27	158
	distal cortex	R	2.71	2.00	43	117
		L	1.96	2.70	31	156
	proximal marrow	R	32.38	26.95	158	484
		L	33.83	25.76	165	463
	distal marrow	R	5.52	9.53	25	158
		L	6.97	5.52	28	82
	skeletal muscle	R	5.00	3.72	43	117
		L	5.29	2.68	41	76
5	proximal cortex	R	1.01	1.29	58	95
		L	0.43	0.99	25	73
	distal cortex	R	1.57	1.67	81	110
		L	1.53	1.73	76	110
	proximal marrow	R	48.42	54.91	568	825
		L	43.26	37.17	507	558
	distal marrow	R	18.68	15.56	187	200
		L	15.77	10.77	153	134
	skeletal muscle	R	11.17	13.13	287	433
		L	8.26	8.79	305	416

Table A.3.4 (continued)

No.			Flow		Ms number	
			16.5μm	11.3μm	16.5μm	11.3μm
6	proximal cortex	R	0.81	0.69	16	91
		L	1.01	1.01	19	127
	distal cortex	R	0.97	1.35	16	144
		L	1.19	1.49	19	161
	proximal marrow	R	37.59	23.56	157	654
		L	37.37	33.22	149	883
	distal marrow	R	13.34	11.60	51	295
		L	13.27	15.64	45	351
	skeletal muscle	R	22.74	19.04	250	1394
		L	28.10	23.51	299	1666

Table A.3.5   Section 2.4   Blood flows to the tibial diaphysis and muscle of the anterior compartment, measured sequentially with 11.3 ± 0.1 (SD) μm microspheres. The dimension of flow is ml/min.100g.

No.			Flow First	Flow Second	Ms number First	Ms number Second
1	proximal cortex	R	1.95	3.69	159	99
		L	1.52	2.00	140	60
	distal cortex	R	2.95	3.37	223	84
		L	2.56	3.99	198	101
	proximal marrow	R	34.82	64.17	643	390
		L	30.97	49.11	609	317
	distal marrow	R	19.55	28.64	277	133
		L	12.63	22.68	194	114
	skeletal muscle	R	11.57	5.98	341	58
		L	11.78	7.88	333	73
2	proximal cortex	R	3.34	3.47	136	310
		L	3.66	3.96	154	368
	distal cortex	R	3.88	4.84	135	370
		L	4.99	5.35	187	440
	proximal marrow	R	31.19	46.33	351	1148
		L	35.74	48.73	395	1186
	distal marrow	R	15.98	25.55	125	441
		L	13.54	25.34	114	471
	skeletal muscle	R	22.53	11.77	371	427
		L	25.50	10.70	379	350

Table A.3.5 (continued)

No.			Flow		Ms number	
			First	Second	First	Second
3	proximal cortex	R	3.13	3.55	395	181
		L	3.46	4.44	430	223
	distal cortex	R	4.26	5.72	463	251
		L	3.72	4.61	402	201
	proximal marrow	R	66.56	118.03	1717	1229
		L	64.63	109.42	1580	1079
	distal marrow	R	27.72	44.81	809	528
		L	27.43	40.97	745	449
	skeletal muscle	R	5.28	5.78	269	119
		L	7.20	6.54	288	106
4	proximal cortex	R	1.83	0.97	72	102
		L	1.38	0.92	53	95
	distal cortex	R	1.94	0.99	66	89
		L	1.50	1.31	50	117
	proximal marrow	R	76.83	42.23	581	850
		L	79.35	44.13	585	866
	distal marrow	R	13.28	11.23	73	165
		L	11.24	9.27	60	132
	skeletal muscle	R	6.69	3.02	84	101
		L	4.68	1.87	86	92
5	proximal cortex	R	3.58	3.42	531	229
		L	1.66	1.96	205	109
	distal cortex	R	1.58	1.36	181	70
		L	1.31	1.59	142	77
	proximal marrow	R	42.54	53.72	1326	753
		L	37.16	49.69	1029	620
	distal marrow	R	26.71	25.02	570	240
		L	17.09	18.93	335	167
	skeletal muscle	R	9.81	8.67	521	207
		L	6.01	9.18	177	122

Table A.3.5 (continued)

No.			Flow		Ms number	
			First	Second	First	Second
6	proximal cortex	R	0.33	0.83	60	205
		L	0.37	0.80	64	185
	distal cortex	R	0.35	0.92	55	195
		L	0.49	0.94	75	194
	proximal marrow	R	5.82	13.11	248	754
		L	5.70	12.86	222	676
	distal marrow	R	1.97	4.41	78	237
		L	1.63	4.88	56	226
	skeletal muscle	R	2.97	4.33	171	336
		L	2.06	2.41	114	181

Table A.3.6 Section 2.5 One week group. Blood flows to the tibial diaphysis and muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres before and after immobilisation of one hindlimb in a cast for one week. The dimension of flow is ml/min.100g.

No.			Flow Before	After	Ms number Before	After
1	proximal cortex	E	2.35	1.31	110	53
		C	4.96	2.25	237	92
	distal cortex	E	1.12	0.69	47	24
		C	1.85	0.96	73	33
	proximal marrow	E	45.77	23.37	389	170
		C	35.23	22.43	291	159
	distal marrow	E	0.94	1.06	5	4
		C	1.12	0.76	6	4
	skeletal muscle	E	8.92	6.14	162	96
		C	8.58	4.23	179	76
2	proximal cortex	E	0.48	0.23	24	64
		C	1.24	0.37	59	99
	distal cortex	E	0.35	0.13	15	30
		C	0.35	0.14	16	36
	proximal marrow	E	10.72	7.11	104	385
		C	11.14	6.98	101	352
	distal marrow	E	1.28	1.25	10	52
		C	1.55	1.03	11	41
	skeletal muscle	E	2.41	9.86	53	1218
		C	2.81	4.76	53	499

Table A.3.6 (continued)

No.			Flow		Ms number	
			Before	After	Before	After
3	proximal cortex	E	0.76	0.45	69	26
		C	0.95	0.51	88	30
	distal cortex	E	1.24	0.95	98	47
		C	1.28	0.75	103	38
	proximal marrow	E	23.21	18.85	410	211
		C	25.71	18.63	454	208
	distal marrow	E	13.83	10.12	199	92
		C	13.42	9.45	181	81
	skeletal muscle	E	7.97	4.99	308	122
		C	5.48	5.96	229	158
4	proximal cortex	E	0.65	0.25	18	38
		C	1.07	0.40	28	56
	distal cortex	E	1.82	0.42	38	48
		C	0.33	0.10	8	12
	proximal marrow	E	35.98	14.76	212	469
		C	42.70	17.84	229	516
	distal marrow	E	7.34	3.09	37	85
		C	5.85	1.51	31	43
	skeletal muscle	E	9.77	11.72	134	869
		C	4.70	6.59	39	293
5	proximal cortex	E	0.55	0.68	153	158
		C	0.44	0.49	116	109
	distal cortex	E	0.64	0.92	148	176
		C	0.58	0.77	127	140
	proximal marrow	E	8.83	23.29	621	1359
		C	6.23	18.30	382	930
	distal marrow	E	1.73	12.19	83	488
		C	0.96	8.45	42	311
	skeletal muscle	E	2.90	4.88	196	274
		C	2.41	5.77	163	325

Table A.3.6 (continued)

No.			Flow		Ms number	
			Before	After	Before	After
6	proximal cortex	E	3.53	0.59	171	178
		C	3.43	0.58	170	177
	distal cortex	E	4.16	0.58	173	149
		C	2.87	0.51	115	126
	proximal marrow	E	44.57	18.13	578	1456
		C	41.76	19.50	562	1623
	distal marrow	E	4.76	2.04	43	113
		C	4.30	2.69	38	145
	skeletal muscle	E	4.37	8.58	66	802
		C	4.81	7.80	114	1139

Table A.3.7   Section 2.5   Two week group.   Blood flows to the tibial diaphysis and muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres before and after immobilisation of one hindlimb in a cast for two weeks. The dimension of flow is ml/min.100g.

No.			Flow Before	After	Ms number Before	After
1	proximal cortex	E	1.09	0.83	109	90
		C	1.00	0.78	107	90
	distal cortex	E	0.88	0.79	82	80
		C	1.02	0.80	93	79
	proximal marrow	E	20.18	34.04	452	828
		C	21.73	31.65	526	832
	distal marrow	E	4.23	5.02	77	99
		C	4.48	5.36	76	99
	skeletal muscle	E	3.24	17.94	98	590
		C	4.95	12.04	144	380
2	proximal cortex	E	1.10	0.36	75	147
		C	1.34	0.45	86	170
	distal cortex	E	0.85	0.24	48	80
		C	0.99	0.25	56	84
	proximal marrow	E	24.29	8.40	506	1038
		C	26.27	9.02	532	1083
	distal marrow	E	5.50	3.33	88	316
		C	5.84	3.61	90	330
	skeletal muscle	E	3.86	2.47	92	350
		C	2.77	2.63	54	301

Table A.3.7 (continued)

No.			Flow		Ms number	
			Before	After	Before	After
3	proximal cortex	E	3.08	1.34	266	105
		C	2.71	1.74	223	129
	distal cortex	E	3.26	1.79	253	125
		C	3.44	1.61	252	106
	proximal marrow	E	31.44	36.98	664	705
		C	30.57	38.91	634	729
	distal marrow	E	7.23	9.68	115	138
		C	6.26	12.22	90	158
	skeletal muscle	E	10.78	12.05	305	308
		C	7.09	5.38	201	137
4	proximal cortex	E	0.46	3.09	62	278
		C	1.62	3.14	234	303
	distal cortex	E	1.21	3.81	139	292
		C	0.48	1.51	57	121
	proximal marrow	E	34.60	42.14	1131	920
		C	30.94	47.05	869	883
	distal marrow	E	12.67	8.40	298	132
		C	8.56	6.59	172	88
	skeletal muscle	E	8.11	9.47	335	261
		C	7.44	9.63	269	232
5	proximal cortex	E	2.25	0.85	236	126
		C	2.62	0.88	267	126
	distal cortex	E	2.50	0.74	228	95
		C	2.12	0.64	197	83
	proximal marrow	E	15.35	28.74	415	1095
		C	17.61	23.20	449	832
	distal marrow	E	4.32	10.66	85	295
		C	2.57	5.20	52	148
	skeletal muscle	E	13.37	4.42	447	208
		C	9.95	5.68	375	301

Table A.3.7 (continued)

No.			Flow		Ms number	
			Before	After	Before	After
6	proximal cortex	E	1.18	1.67	190	320
		C	1.13	1.42	192	286
	distal cortex	E	1.05	1.00	148	168
		C	0.97	1.05	137	176
	proximal marrow	E	14.80	49.90	533	2131
		C	17.03	45.78	693	2210
	distal marrow	E	3.24	11.55	114	482
		C	3.04	6.72	105	274
	skeletal muscle	E	4.65	5.61	306	438
		C	5.28	5.32	343	410

Table A.3.8 Section 3.1 Control group, one week subgroup.
Blood flows to the tibial diaphysis and the muscle of the
anterior compartment, measured with 11.3 ± 0.1 (SD) μm
microspheres one week after a unilateral tibial diaphyseal
osteotomy. The dimension of flow is ml/min.100g.

No.			Flow	Ms number
1	proximal cortex	E	2.13	435
		C	0.83	168
	distal cortex	E	1.70	270
		C	0.85	138
	proximal marrow	E	29.02	1427
		C	19.01	1008
	distal marrow	E	7.12	268
		C	2.86	88
	skeletal muscle	E	8.78	609
		C	4.37	240
2	proximal cortex	E	5.02	470
		C	2.92	274
	distal cortex	E	3.26	206
		C	3.27	220
	proximal marrow	E	73.99	1747
		C	62.95	1608
	distal marrow	E	15.54	235
		C	18.76	276
	skeletal muscle	E	16.32	588
		C	21.35	711

Table A.3.8 (continued)

No.			Flow	Ms number
3	proximal cortex	E	3.11	384
		C	1.86	192
	distal cortex	E	1.71	166
		C	1.56	153
	proximal marrow	E	35.07	1137
		C	25.22	727
	distal marrow	E	11.20	289
		C	8.96	242
	skeletal muscle	E	18.82	780
		C	16.35	668
4	proximal cortex	E	2.74	1309
		C	0.77	428
	distal cortex	E	2.22	1078
		C	0.64	230
	proximal marrow	E	16.75	2008
		C	11.34	1388
	distal marrow	E	7.64	1052
		C	5.29	486
	skeletal muscle	E	8.83	1396
		C	3.17	462
5	proximal cortex	E	1.94	1047
		C	1.09	608
	distal cortex	E	0.89	443
		C	0.70	345
	proximal marrow	E	16.08	2927
		C	19.69	3535
	distal marrow	E	4.37	753
		C	8.28	1186
	skeletal muscle	E	7.69	1642
		C	12.23	1306

Table A.3.8 (continued)

No.			Flow	Ms number
6	proximal cortex	E	1.62	477
		C	1.02	276
	distal cortex	E	0.96	181
		C	3.96	214
	proximal marrow	E	23.71	1632
		C	23.55	1759
	distal marrow	E	5.94	339
		C	10.30	574
	skeletal muscle	E	7.25	488
		C	3.48	255

Table A.3.9 Section 3.1 Control group, two week subgroup. Blood flows to the tibial diaphysis and the muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres two weeks after a unilateral tibial diaphyseal osteotomy. The dimension of flow is ml/min.100g.

No.			Flow	Ms number
1	proximal cortex	E	10.25	1690
		C	1.87	274
	distal cortex	E	5.69	847
		C	3.03	381
	proximal marrow	E	40.80	1403
		C	41.82	1497
	distal marrow	E	17.11	432
		C	30.45	855
	skeletal muscle	E	8.88	461
		C	5.92	216
2	proximal cortex	E	5.09	1552
		C	1.39	364
	distal cortex	E	5.06	1260
		C	1.17	215
	proximal marrow	E	33.13	1706
		C	29.45	1886
	distal marrow	E	11.61	453
		C	6.81	313
	skeletal muscle	E	19.44	1976
		C	11.82	1053

Table A.3.9 (continued)

No.			Flow	Ms number
3	proximal cortex	E	4.27	2992
		C .	0.78	466
	distal cortex	E	3.96	1660
		C	0.58	239
	proximal marrow	E	22.96	2950
		C	19.72	2864
	distal marrow	E	11.72	1408
		C	5.61	658
	skeletal muscle	E	4.87	1074
		C	1.51	337
4	proximal cortex	E	5.83	731
		C	2.72	243
	distal cortex	E	7.21	690
		C	1.94	157
	proximal marrow	E	21.62	410
		C	42.90	920
	distal marrow	E	16.66	357
		C	10.75	186
	skeletal muscle	E	18.13	711
		C	8.10	274
5	proximal cortex	E	2.55	594
		C	1.01	196
	distal cortex	E	3.48	658
		C	1.07	164
	proximal marrow	E	31.54	1554
		C	13.87	1927
	distal marrow	E	8.86	373
		C	7.15	269
	skeletal muscle	E	9.98	832
		C	4.72	377

Table A.3.9 (continued)

No.			Flow	Ms number
6	proximal cortex	E	7.35	1452
		C	4.29	558
	distal cortex	E	9.67	1410
		C	4.16	450
	proximal marrow	E	18.41	736
		C	57.02	2459
	distal marrow	E	16.46	555
		C	27.98	1009
	skeletal muscle	E	17.40	818
		C	6.88	502

Table A.3.10 Section 3.1 Sheath group, one week subgroup. Blood flows to the tibial diaphysis and the muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres one week after a unilateral tibial diaphyseal osteotomy with the periosteum and soft tissues excluded by a silastic sheath. The dimension of flow is ml/min.100g.

No.			Flow	Ms number
1	proximal cortex	E	3.40	415
		C	3.06	361
	distal cortex	E	1.52	163
		C	1.62	176
	proximal marrow	E	33.07	775
		C	28.52	686
	distal marrow	E	8.38	201
		C	5.54	120
	skeletal muscle	E	11.91	716
		C	5.49	208
2	proximal cortex	E	0.37	106
		C	0.24	73
	distal cortex	E	0.29	83
		C	0.17	48
	proximal marrow	E	13.63	968
		C	13.09	1116
	distal marrow	E	5.40	315
		C	4.30	287
	skeletal muscle	E	6.40	700
		C	4.60	451

Table A.3.10 (continued)

No.			Flow	Ms number
3	proximal cortex	E	1.92	739
		C	1.16	414
	distal cortex	E	0.47	151
		C	0.36	111
	proximal marrow	E	17.15	1769
		C	19.39	1681
	distal marrow	E	2.96	234
		C	1.64	113
	skeletal muscle	E	1.76	132
		C	1.01	270
4	proximal cortex	E	1.11	170
		C	1.01	145
	distal cortex	E	2.14	242
		C	1.59	175
	proximal marrow	E	18.34	787
		C	27.85	1108
	distal marrow	E	8.94	293
		C	10.21	335
	skeletal muscle	E	17.22	478
		C	18.99	887
5	proximal cortex	E	0.56	182
		C	0.71	226
	distal cortex	E	0.53	136
		C	0.44	114
	proximal marrow	E	9.77	854
		C	10.64	946
	distal marrow	E	2.45	149
		C	2.99	200
	skeletal muscle	E	6.83	586
		C	4.95	455

Table A.3.10 (continued)

No.			Flow	Ms number
6	proximal cortex	E	0.33	141
		C	0.31	125
	distal cortex	E	0.37	109
		C	0.33	96
	proximal marrow	E	6.41	729
		C	5.91	599
	distal marrow	E	1.77	103
		C	3.07	184
	skeletal muscle	E	1.28	177
		C	2.03	236

Table A.3.11   Section 3.1   Sheath group, two week subgroup. Blood flows to the tibial diaphysis and the muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres two weeks after a unilateral tibial diaphyseal osteotomy with the periosteum and soft tissues excluded by a silastic sheath. The dimension of flow is ml/min.100g.

No.			Flow	Ms number
1	proximal cortex	E	1.96	240
		C	2.23	261
	distal cortex	E	1.85	169
		C	1.00	90
	proximal marrow	E	41.83	1214
		C	50.10	1586
	distal marrow	E	21.79	556
		C	19.29	484
	skeletal muscle	E	25.83	591
		C	18.41	559
2	proximal cortex	E	1.45	322
		C	0.50	105
	distal cortex	E	2.62	399
		C	0.75	112
	proximal marrow	E	16.17	898
		C	21.90	1197
	distal marrow	E	8.24	318
		C	9.10	383
	skeletal muscle	E	7.04	504
		C	5.10	260

Table A.3.11 (continued)

No.			Flow	Ms number
3	proximal cortex	E	1.07	399
		C	0.46	138
	distal cortex	E	1.22	266
		C	0.58	124
	proximal marrow	E	15.27	1049
		C	17.52	1181
	distal marrow	E	6.68	270
		C	9.54	386
	skeletal muscle	E	5.48	517
		C	3.94	468
4	proximal cortex	E	3.02	751
		C	6.14	1385
	distal cortex	E	3.14	516
		C	6.45	910
	proximal marrow	E	25.88	1541
		C	43.74	2605
	distal marrow	E	15.51	713
		C	17.99	902
	skeletal muscle	E	7.83	450
		C	3.57	205
5	proximal cortex	E	2.23	387
		C	0.76	105
	distal cortex	E	0.43	56
		C	0.73	83
	proximal marrow	E	37.34	1529
		C	31.75	1300
	distal marrow	E	4.51	137
		C	11.65	311
	skeletal muscle	E	3.14	162
		C	2.08	117

'Table A.3.11 (continued)

No.			Flow	Ms number
6	proximal cortex	E	3.45	627
		C .	1.89	316
	distal cortex	E	1.93	294
		C	1.29	195
	proximal marrow	E	31.74	1361
		C	27.76	1074
	distal marrow	E	20.76	786
		C	7.35	235
	skeletal muscle	E	22.17	951
		C	20.43	1632

Table A.3.12 Section 3.1 Nail group, one week subgroup. Blood flows to the tibial diaphysis and the muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres one week after a unilateral tibial diaphyseal osteotomy with intramedullary nailing. The dimension of flow is ml/min.100g.

No.			Flow	Ms number
1	proximal cortex	E	0.41	82
		C	0.56	102
	distal cortex	E	0.49	56
		C	0.66	74
	proximal marrow	E	9.69	160
		C	19.56	760
	distal marrow	E	1.24	13
		C	8.45	237
	skeletal muscle	E	15.58	897
		C	7.57	501
2	proximal cortex	E	1.66	141
		C	2.42	210
	distal cortex	E	1.27	112
		C	1.88	166
	proximal marrow	E	19.12	237
		C	44.46	1081
	distal marrow	E	14.92	177
		C	22.74	541
	skeletal muscle	E	20.44	677
		C	17.08	671

Table A.3.12 (continued)

No.			Flow	Ms number
3	proximal cortex	E	5.61	941
		C	3.26	464
	distal cortex	E	10.60	1364
		C	6.13	660
	proximal marrow	E	5.93	116
		C	34.58	1474
	distal marrow	E	5.11	107
		C	8.24	303
	skeletal muscle	E	8.07	291
		C	12.10	700
4	proximal cortex	E	7.79	827
		C	4.02	353
	distal cortex	E	10.13	925
		C	5.62	433
	proximal marrow	E	76.23	1051
		C	125.25	2621
	distal marrow	E	65.19	1131
		C	47.99	1127
	skeletal muscle	E	10.89	200
		C	16.14	577
5	proximal cortex	E	4.40	572
		C	2.31	275
	distal cortex	E	6.07	500
		C	3.14	238
	proximal marrow	E	52.90	902
		C	75.52	1864
	distal marrow	E	12.20	194
		C	26.79	598
	skeletal muscle	E	18.05	583
		C	29.39	1261

Table A.3.12 (continued)

No.			Flow	Ms number
6	proximal cortex	E	3.24	542
		C	2.43	389
	distal cortex	E	4.74	612
		C	4.00	466
	proximal marrow	E	20.78	386
		C	55.51	1968
	distal marrow	E	10.69	289
		C	13.93	470
	skeletal muscle	E	17.82	917
		C	6.14	326

Table A.3.13 Section 3.1 Nail group, two week subgroup. Blood flows to the tibial diaphysis and the muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) µm microspheres two weeks after a unilateral tibial diaphyseal osteotomy with intramedullary nailing. The dimension of flow is ml/min.100g.

No.			Flow	Ms number
1	proximal cortex	E	2.71	431
		C	2.27	246
	distal cortex	E	5.01	696
		C	2.80	257
	proximal marrow	E	30.33	219
		C	68.17	1744
	distal marrow	E	17.75	244
		C	23.70	529
	skeletal muscle	E	10.93	301
		C	8.99	301
2	proximal cortex	E	1.40	733
		C	1.25	364
	distal cortex	E	1.85	675
		C	1.35	308
	proximal marrow	E	1.58	93
		C	21.82	1928
	distal marrow	E	9..68	525
		C	4.04	307
	skeletal muscle	E	5.52	428
		C	6.39	654

Table A.3.13 (continued)

No.			Flow	Ms number
3	proximal cortex	E	5.14	718
		C	1.82	181
	distal cortex	E	7.13	796
		C	4.16	310
	proximal marrow	E	63.41	1031
		C	86.46	2422
	distal marrow	E	78.02	1057
		C	30.83	669
	skeletal muscle	E	28.55	787
		C	19.36	691
4	proximal cortex	E	4.48	1660
		C	1.27	412
	distal cortex	E	6.43	2252
		C	1.22	326
	proximal marrow	E	6.27	377
		C	40.95	4347
	distal marrow	E	3.39	86
		C	9.06	646
	skeletal muscle	E	10.87	1103
		C	5.57	742
5	proximal cortex	E	1.55	836
		C	1.63	646
	distal cortex	E	2.13	870
		C	1.01	346
	proximal marrow	E	0.98	44
		C	45.08	4832
	distal marrow	E	12.73	635
		C	25.49	2351
	skeletal muscle	E	5.94	815
		C	2.35	481

Table A.3.13 (continued)

No.			Flow	Ms number
6	proximal cortex	E	1.01	594
		C	0.47	223
	distal cortex	E	1.57	832
		C	0.18	74
	proximal marrow	E	4.55	397
		C	28.02	4086
	distal marrow	E	2.28	144
		C	13.86	1568
	skeletal muscle	E	7.19	1253
		C	4.64	939

Table A.3.14 Section 3.2 Lower energy group, one week subgroup. Blood flows to the tibial diaphysis and the muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres one week after a unilateral lower energy tibial diaphyseal fracture. The dimension of flow is ml/min.100g.

No.			Flow	Ms number
1	proximal cortex	E	4.01	1553
		C	2.58	892
	distal cortex	E	1.87	983
		C	1.91	912
	proximal marrow	E	7.95	804
		C	24.42	2577
	distal marrow	E	2.51	309
		C	11.97	1684
	skeletal muscle	E	21.02	2587
		C	0.99	100
2	proximal cortex	E	3.64	925
		C	2.75	619
	distal cortex	E	5.76	679
		C	5.27	561
	proximal marrow	E	35.16	1450
		C	60.41	2541
	distal marrow	E	9.42	233
		C	23.77	667
	skeletal muscle	E	8.56	423
		C	3.57	147

Table A.3.14 (continued)

No.			Flow	Ms number
3	proximal cortex	E	2.66	813
		C	2.08	510
	distal cortex	E	6.23	1068
		C	5.40	667
	proximal marrow	E	8.24	538
		C	28.14	2040
	distal marrow	E	5.37	241
		C	12.18	522
	skeletal muscle	E	8.35	545
		C	3.42	185
4	proximal cortex	E	0.80	508
		C	0.45	264
	distal cortex	E	1.05	370
		C	1.25	413
	proximal marrow	E	11.42	1534
		C	17.97	2548
	distal marrow	E	3.52	307
		C	4.76	427
	skeletal muscle	E	8.63	1138
		C	4.89	474
5	proximal cortex	E	0.75	1185
		C	0.39	567
	distal cortex	E	0.95	672
		C	0.71	442
	proximal marrow	E	6.82	1971
		C	6.71	1865
	distal marrow	E	2.27	303
		C	4.44	688
	skeletal muscle	E	2.12	929
		C	1.08	352

Table A.3.14 (continued)

No.			Flow	Ms number
6	proximal cortex	E	0.73	586
		C	0.41	292
	distal cortex	E	2.00	717
		C	0.75	230
	proximal marrow	E	4.83	884
		C	5.81	1536
	distal marrow	E	1.59	162
		C	3.76	488
	skeletal muscle	E	2.32	377
		C	3.39	621

Table A.3.15   Section 3.2   Lower energy group, two week
subgroup.   Blood flows to the tibial diaphysis and the
muscle of the anterior compartment, measured with
11.3 ± 0.1   (SD)   μm microspheres two weeks after a
unilateral lower energy tibial diaphyseal fracture. The
dimension of flow is ml/min.100g.

No.			Flow	Ms number
1	proximal cortex	E	2.30	1248
		C	1.44	558
	distal cortex	E	4.17	1249
		C	3.55	669
	proximal marrow	E	41.54	3075
		C	41.42	3764
	distal marrow	E	10.32	503
		C	8.56	518
	skeletal muscle	E	2.27	206
		C	3.50	342
2	proximal cortex	E	0.42	1072
		C	0.32	547
	distal cortex	E	2.16	3291
		C	0.89	848
	proximal marrow	E	6.27	2354
		C	4.70	2373
	distal marrow	E	2.27	662
		C	2.63	801
	skeletal muscle	E	1.21	369
		C	0.27	158

Table A.3.15 (continued)

No.			Flow	Ms number
3	proximal cortex	E	2.01	1103
		C	0.65	254
	distal cortex	E	7.22	1630
		C	3.07	540
	proximal marrow	E	24.82	2467
		C	29.06	2888
	distal marrow	E	7.45	516
		C	12.01	796
	skeletal muscle	E	13.66	761
		C	3.07	263
4	proximal cortex	E	2.95	1130
		C	1.60	420
	distal cortex	E	0.84	476
		C	0.46	222
	proximal marrow	E	6.55	546
		C	19.31	1726
	distal marrow	E	5.46	662
		C	7.09	944
	skeletal muscle	E	3.28	352
		C	3.42	578
5	proximal cortex	E	0.90	738
		C	0.32	218
	distal cortex	E	2.90	1102
		C	1.12	392
	proximal marrow	E	19.46	2340
		C	16.82	2164
	distal marrow	E	7.45	521
		C	8.66	678
	skeletal muscle	E	7.32	1105
		C	3.15	554

Table A.3.15 (continued)

No.			Flow	Ms number
6	proximal cortex	E	3.18	794
		C	0.69	151
	distal cortex	E	5.97	998
		C	1.53	173
	proximal marrow	E	25.71	522
		C	33.63	1004
	distal marrow	E	13.72	229
		C	19.28	437
	skeletal muscle	E	28.28	1756
		C	3.48	266

Table A.3.16 Section 3.2 Higher energy group, one week subgroup. Blood flows to the tibial diaphysis and the muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres one week after a unilateral higher energy tibial diaphyseal fracture. The dimension of flow is ml/min.100g.

No.			Flow	Ms number
1	proximal cortex	E	0.71	600
		C	0.37	281
	distal cortex	E	2.71	1179
		C	1.86	791
	proximal marrow	E	12.22	2099
		C	16.52	2946
	distal marrow	E	6.44	731
		C	11.11	1477
	skeletal muscle	E	6.61	1029
		C	3.63	648
2	proximal cortex	E	1.31	405
		C	0.55	170
	distal cortex	E	6.78	1279
		C	2.94	498
	proximal marrow	E	36.03	2544
		C	74.27	5064
	distal marrow	E	11.06	431
		C	23.28	1134
	skeletal muscle	E	1.19	240
		C	3.48	170

Table A.3.16 (continued)

No.			Flow	Ms number
3	proximal cortex	E	0.51	238
		C	0.40	188
	distal cortex	E	2.03	499
		C	1.71	413
	proximal marrow	E	19.14	2327
		C	31.66	3849
	distal marrow	E	8.76	732
		C	9.35	764
	skeletal muscle	E	2.83	317
		C	1.77	289
4	proximal cortex	E	0.26	170
		C	0.14	82
	distal cortex	E	1.20	455
		C	0.68	213
	proximal marrow	E	0.14	26
		C	11.76	1752
	distal marrow	E	2.49	294
		C	4.52	465
	skeletal muscle	E	2.10	400
		C	1.59	395
5	proximal cortex	E	0.55	427
		C	0.47	333
	distal cortex	E	1.58	674
		C	1.04	396
	proximal marrow	E	14.48	1972
		C	21.52	3155
	distal marrow	E	8.92	841
		C	7.53	788
	skeletal muscle	E	4.44	1162
		C	2.94	759

Table A.3.17 Section 3.2 Higher energy group, two week subgroup. Blood flows to the tibial diaphysis and the muscle of the anterior compartment, measured with 11.3 ± 0.1 (SD) μm microspheres two weeks after a unilateral higher energy tibial diaphyseal fracture. The dimension of flow is ml/min.100g.

No.			Flow	Ms number
1	proximal cortex	E	3.81	1587
		C	0.94	295
	distal cortex	E	14.10	2451
		C	4.21	568
	proximal marrow	E	41.32	2612
		C	42.03	3065
	distal marrow	E	24.34	799
		C	11.97	495
	skeletal muscle	E	7.50	428
		C	5.27	423
2	proximal cortex	E	0.58	353
		C	0.20	96
	distal cortex	E	2.43	810
		C	0.74	179
	proximal marrow	E	26.14	3366
		C	27.15	3869
	distal marrow	E	4.87	444
		C	9.36	932
	skeletal muscle	E	2.59	200
		C	2.04	140

Table A.3.17 (continued)

No.			Flow	Ms number
3	proximal cortex	E	1.15	618
		C	0.73	289
	distal cortex	E	5.98	1487
		C	2.08	383
	proximal marrow	E	57.55	5552
		C	48.73	4407
	distal marrow	E	13.87	753
		C	14.40	847
	skeletal muscle	E	8.72	697
		C	4.32	372
4	proximal cortex	E	0.91	377
		C	0.55	177
	distal cortex	E	2.56	560
		C	1.47	269
	proximal marrow	E	18.31	1274
		C	19.63	1392
	distal marrow	E	5.43	238
		C	6.35	278
	skeletal muscle	E	4.28	293
		C	3.40	307
5	proximal cortex	E	1.43	655
		C	0.66	255
	distal cortex	E	4.25	1305
		C	2.54	590
	proximal marrow	E	35.46	2830
		C	18.90	1574
	distal marrow	E	14.62	913
		C	5.94	392
	skeletal muscle	E	4.10	434
		C	2.24	230

Table A.3.17 (continued)

No.			Flow	Ms number
6	proximal cortex	E	1.61	682
		C	0.56	214
	distal cortex	E	7.38	1756
		C	2.93	521
	proximal marrow	E	46.50	3067
		C	53.59	3996
	distal marrow	E	13.41	539
		C	16.27	793
	skeletal muscle	E	2.71	198
		C	1.03	87

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