DELAYED UNION OF FRACTURES OF THE SHAFT OF THE ADULT TIBIA:

A CLINICAL AND EXPERIMENTAL STUDY.

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Delayed Union of Fractures of the Shaft of the Adult Tibia: A clinical and experimental study.

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Abstract

Fractures of the adult tibial shaft are common and often result in considerable morbidity because a significant number exhibit slow healing. The reasons for this are not known and, therefore, prevention is not possible. This has been investigated using clinical and experimental methods. One hundred closed adult tibial shaft fractures treated by closed methods were studied to determine the true delayed union rate and the clinical factors that may be of importance. In addition, an attempt was made to predict fractures at risk using serial biochemical measurements and scintigraphic examination using technecium 99m methylene diphosphonate. The patterns of soft tissue damage in tibial shaft fractures were investigated experimentally in rabbits. Healing of a simple osteotomy was studied after the exclusion of periosteum, medullary or both tissues respectively from the fracture site. Cortical arterial perfusion was studied after the isolation of periosteal, nutrient and epiphyseo-metaphyseal circulations respectively using a diaphyseal segment model and barium sulphate perfusion techniques. The delayed union rate was 19% at 20 weeks and 15 more fractures united at 30 weeks with continued conservative treatment. Of the clinical factors investigated, only the severity of trauma appeared to play a part in delayed union. Serum creatinine phosphokinase levels rose after fracture and provided some indication of severity of trauma. Serum calcium and inorganic phosphate levels rose after fracture and then progressively fell over a 20 week period. Serum levels of osteocalcin and somatomedins did not fluctuate. Biochemical parameters were not useful in identifying fractures at risk of developing delayed union. Of all scintigraphic methods, only the A/C ratio of uptake over the fracture site relative to an adjacent site, with a cut off value of 2.0, clearly separated normal from delayed union. Simple osteotomies of the rabbit tibial diaphysis healed normally in the absence of medullary tissues but not in the absence of periosteum. Cortical arterial perfusion was observed in diaphyseal segments of the rabbit tibial diaphysis up to 2 weeks but not thereafter when entrusted to the nutrient circulation alone. When entrusted to the periosteal circulation alone, cortical perfusion could be observed immediately after death, but not thereafter until 2 weeks after the procedure had been performed.
To the memory of my father,
David Babajide Oni B.A Hon. (Lond.)
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PREFACE

The rapid increase in the number of motor vehicles and cycles and mass participation in various sports have resulted in an ever increasing number of people sustaining fractures of the tibial shaft. The Leicester Trauma and Orthopaedic Service currently can expect to treat approximately 200 adults with this fracture every year.

Adult tibial shaft fractures generate more controversy than most other fractures presumably because they are common and, therefore, all grades of orthopaedic surgeons have some experience in their treatment. However, clinical experience suggests that these are often not easy fractures to treat and in particular, a significant proportion of them do not unite on time.

As a consequence of delayed union, otherwise fit and active young people may suffer considerable morbidity for a long period of time after fracture. Their services are usually lost to the labour force during this period and they may require social security assistance.

Unfortunately, the exact aetiology of delayed union is not known and, therefore, methods of prevention have not been determined. For this reason, early diagnosis is of paramount importance so that appropriate action may be taken in an attempt to reduce the overall morbidity.
A number of methods are used almost intuitively to identify fractures at risk of developing delayed union. For example, fractures are classified as closed or open because in most surgeons' experience, open fractures heal more slowly than closed ones. Fracture behaviour is also inferred from the radiographic features. Comminuted and segmental fractures, for example, appear to be slower to heal than simple fractures. These methods are unreliable, however, because they are not able to accurately forecast the fate of individual fractures.

Knowledge of the overall natural history of individual types of adult tibial shaft fracture is crucial but this is far from complete. Many studies attempt too ambitiously to include all potentially important variables and consequently, the results are reduced to statistical insignificance.

This thesis reports the results of investigations carried out in relation to the early diagnosis and aetiology of delayed union of adult tibial shaft fractures. Experimental studies have been carried out in an attempt to identify the critical tissues and/or critical blood supply in fracture healing.

The studies were performed during the period of 1 August, 1985 and 31 July, 1987 while the author held the post of Lecturer in the University Department of Orthopaedic Surgery, Leicester.
I should like to acknowledge the support of all the Consultant Orthopaedic Surgeons at the Leicester Royal Infirmary who allowed me to study their patients. I am also indebted to my other colleagues in the Department of Orthopaedic Surgery who ensured that the appropriate initial investigations of patients were carried out and that patients were referred promptly to the Tibial Fracture Study Clinic. Sister Hazel Richards and her Staff ensured the smooth running of the clinic and Margaret Steward and her staff were responsible for making appointments and for looking after case files.

I am grateful to Dr. J. Iqbal, consultant in the Department of Clinical Chemistry, Leicester Royal Infirmary, for his help in interpreting the biochemistry results. John Mahabir and Dave Valance, the chief M.L.S.Os. performed the osteocalcin and somatomedin assays respectively.

I am indebted to Mr. M.Y. Early, the Principal Physicist, Leicester Royal Infirmary, who helped administratively to set up the scintigraphic study. Ann Graebe, Senior Physicist, collaborated with me on the study and assisted in interpreting the results.
I am also indebted to many people without whom the experimental work could not have been performed. In particular I am grateful to Dr. D.B. Morton, Director of the Biomedical Services Unit, University of Leicester, for his help in setting up the animal experiments. The success of this work has been partly due to his enthusiasm and the expert care of animals provided by his team. He also assisted in my application for an animal license. Linda Reid and Dr. Jenny Rees were responsible for most of the theatre preparations and animal anaesthesia.

I should like especially to thank Hilary Stafford, Chief Technician, University Department of Orthopaedic Surgery, Glenfield General Hospital, Leicester, for preparing all the specimens and for doubling up as radiographer and photographer. She helped me with the design of the laboratory and with the purchase of the equipment.

I am grateful to Dr. A. Malcolm, Senior Lecturer in Osteo-articular Pathology, University of Newcastle-upon-Tyne, for his help in the interpretation of the histological sections. Professor M. Brookes, Professor of Orthopaedic Anatomy at Guy's Hospital Medical School, London, shared his considerable knowledge of the problems associated with vascular perfusion and provided much useful general advice during the early stages of this work.
I should like to acknowledge the assistance of Zimmer (U.K.) Ltd. who provided a micro-saw with air-hose free-of-charge. The mammography films were donated by 3M Health Care.

The photographic prints and artwork were provided by John Grenfell and his team at the Medical Illustration Unit, Leicester Royal Infirmary. The microradiographs were developed at the X-ray Department of Glenfield General Hospital. Mr. I.H. Thomas, Senior Lecturer, Department of Orthopaedic Surgery, University of Leicester, helped with the selection of the appropriate illustrations.

All computing and statistical work were carried out at the Computer Centre, University of Leicester. The interpretation of data was carried out with the help of J.L. Beckett, Application Programmer, at the centre.

Last but not least, I owe a considerable debt to Professor P.J. Gregg, Professor of Orthopaedic Surgery at the University of Leicester, for giving me the opportunity to undertake this study. The work could not have been carried out successfully without his enthusiastic support and expert supervision. He provided encouragement and advice at every stage of the work. He was also of considerable assistance in the preparation of the text of this thesis, and other publications that have resulted from this work.
"...the best way to treat non union is to prevent its developing..."
Sir John Charnley (1911 - 1982)
1.0 INTRODUCTION.
1.1 ADULT TIBIAL SHAFT FRACTURES

The tibia is one of the commonest long bones to be fractured in adults (Charnley 1970, Wilson 1981, Chapman 1983, Leach 1984). Fractures of the shaft of the tibia are caused by road traffic accidents, accidents at sport and accidents at work or at home. Despite their frequency, however, the methods of treatment are as diverse as they are controversial and the best method of treatment cannot yet be stated with finality (Wilson 1981, Chapman 1983, Leach 1984). This is because the difficulties that confront the surgeon treating fractures of the shaft of the adult tibia are legion. Many of these fractures are open and there is a significant risk of infection which may become chronic (Slattis & Rokkanen 1967). The fracture fragments have a tendency to displace (Sinclair 1931) and there is, therefore, a potential for cosmetic disfigurement. Conservative treatment, using cast immobilisation, may be associated with significant complications such as pressure sores, compartment syndromes and thromboembolic complications, joint stiffness and hindfoot disability (Ellis 1950b) and sympathetic osteodystrophy (Gurd 1934). Operative treatment may be associated with infection (Charnley 1970, Wilson 1981, Chapman 1983, Leach 1984). It may be difficult to know when the fracture is healed which may lead to an unnecessarily long period of semi-protection. In addition, a second operation is usually required to remove the implant(s).
Furthermore, the healing of adult tibial shaft fractures is often delayed (Sinclair 1931, Editorial BMJ 1975) compared to fractures through similar dense cortical bone of the shaft of other long bones (Carpenter et al. 1952, White et al. 1953). The overall prevalence of delayed union has been estimated to be approximately 3% or less in the skeleton as a whole (Blumfeld 1947) but that of the tibia may be as high as 90% in open fractures (Carpenter et al. 1952, Sakellarides et al. 1964). In most reports, including three recent prospective studies (Auchincloss & Watt 1982, Gregg et al. 1983, Haines et al. 1984), union of fractures of the shaft of the adult tibia has been noted to be delayed beyond the accepted 20 weeks in up to a third of cases. Even in the larger reported series, the delayed union rate is from 1 to 17% (Dehne et al. 1961, Nicoll 1964, Edwards 1965a, Weissman et al. 1966, Sarmiento 1967, 1970). Because of this, Souter (1969) advised routine cancellous bone grafting of tibial shaft fractures not united at 12 to 16 weeks.

Delayed union of fractures of the tibial shaft is important because it increases the potential for other complications. Until a tibial shaft fracture has united satisfactorily, the affected lower limb cannot perform its normal function properly without support. In practice, this is usually provided by immobilisation in a plaster of Paris or other suitable cast until union occurs. A major proportion of ununited tibial shaft fractures will eventually heal when treated this way. However, prolonged immobilisation often leads to slow recovery of function (Watson Jones 1943) because immobility encourages muscle atrophy and joint stiffness,
conditions from which it may be difficult to recover without strenuous efforts and supervised rehabilitation. Prolonged immobilisation, by impairing blood and lymphatic circulation in the affected limb may also damage other soft tissues such as the skin which may become atrophic. Consequently, the ability of surgical wounds to heal may be impaired and susceptibility to infection may be increased. The fractured bone becomes porotic and may develop sympathetic osteodystrophy which may take months to resolve. Thus, delayed union results in increased morbidity.

Furthermore, delayed union of tibial shaft fractures also presents financial and social problems for the patient and for his community. The patient, often young and the breadwinner in his family, is frequently unable to fulfill the terms of his employment and, therefore, his salary may be stopped and his family may have to rely on unemployment benefits during the period of incapacity. He may lose his job because his firm would lose his expertise during the time he is off work and be forced to recruit replacement staff.

It is difficult to estimate precisely the costs involved in treating a patient with a fractured tibia because so many variables are involved. The costs vary enormously according to the circumstances of the patient, the fracture type and its treatment and it is, therefore, impossible to arrive at anything more than a very rough idea of the true costs. Nevertheless, the following is an attempt to estimate the cost of a typical case of fractured tibia based on information obtained from various sources including the Leicestershire
Mr. X breaks his leg in a fall. He is brought to hospital by ambulance and examined and radiographed in the Casualty. He is admitted to the wards and taken to theatre where he spends an hour receiving whatever conservative procedures are deemed necessary. He then spends one week as an inpatient. He is discharged to the outpatient clinic for follow-up perhaps every 6 weeks until final discharge at 30 weeks (5 visits) using the ambulance service on each occasion. During the course of treatment, the plaster of Paris cast is changed twice and the leg is radiographed on 7 occasions including the initial visit to the casualty department and one to the theatre. His fractures heal without any complications and Mr. X undertakes physiotherapy for one hour, twice weekly for 6 weeks after cast removal, the first 2 visits by ambulance. He will probably be incapacitated for at least 5 months and spend this period of time (depending on his occupation) on sickness pay. The total costs could amount to:

<table>
<thead>
<tr>
<th>Description</th>
<th>Cost (£)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accident/Emergency department costs</td>
<td>19.00</td>
</tr>
<tr>
<td>Ambulance</td>
<td>96.00</td>
</tr>
<tr>
<td>Inpatient costs (7 days)</td>
<td>721.00</td>
</tr>
<tr>
<td>Theatre costs</td>
<td>60.00</td>
</tr>
<tr>
<td>Outpatient costs - 5 visits</td>
<td>125.00</td>
</tr>
<tr>
<td>Materials</td>
<td>60.00</td>
</tr>
<tr>
<td>Radiographic services</td>
<td>161.00</td>
</tr>
<tr>
<td>Physiotherapy</td>
<td>300.00</td>
</tr>
<tr>
<td>Sickness benefit (£30.05/week for 20 weeks)</td>
<td>601.00</td>
</tr>
<tr>
<td>Total</td>
<td>2143.00</td>
</tr>
</tbody>
</table>
These costs are obviously considerably increased if healing is delayed. Data obtained from the Department of Community Health computer records indicate that approximately 200 cases per annum are treated at the Leicester Royal Infirmary, which caters for between 800,000 and one million population, at an estimated minimum cost of £428,600.

Thus, tibial shaft fractures are common, difficult to treat and are most especially associated with delay in healing. These fractures occur most often in people of working age and are, therefore, costly to the community and to the National Health Service. These fractures pose important problems which deserve study. It is the problem of delayed union that is addressed in this thesis.
1.2 THE BLOOD SUPPLY OF THE SHAFT (DIAPHYSIS) OF THE TIBIA

An adequate blood supply is essential for the viability and growth of bones, and for the pathophysiological changes in response to trauma (Trueta 1968, Brookes 1971). However, the manner in which bone is supplied with blood is the subject of much dispute and it is not possible to give an account of the vascular anatomy of bone which is totally free of controversy.

As long ago as 1691, Havers described how a large nutrient artery pierces the shaft of a long bone to ramify within the marrow in "Osteologia Nova. Some new observations of the bones, communicated to the Royal Society in several discourses". He described little bags of marrow fat, each provided with an arterial stalk which arborised on the surface of the lobule and which secreted an oily medullary substance. He also described a system of straight transcortical pores (Haversian canals) into which he assumed the oily products of the medullary lobules were squirted. Havers believed that the oily products provided suppleness for the cortical lamellae. Finally, Havers described groups of arteries entering the extremities of long bones. He thought that these arteries formed a vascular mesh within the cortex and that this mesh gave rise to several veins leaving the bone at the periosteal surface.

In 1756, Albinus described the results of his injection
techniques. He demonstrated how fine blood vessels were to be found in the tiny canals of the bone cortex. He also surmised that vessels both entered and left the cortex at the internal surface as well as the external surface so that half was supplied from medullary and half from periosteal vessels. By the beginning of the 19th century, it was generally accepted that the internal structures of bone were full of blood and strenuous efforts were made to elucidate the vascular anatomy of bone. Initial anatomical descriptions were mainly based on macroscopic studies of perfused vessels demonstrated by dissection.

The first major description of the vascular anatomy of long bones was given by Langer in 1876. He demonstrated the macroscopic and microscopic vascular anatomy of the human tibia and femur from casts made after injecting dyes and resins into the vessels and marrow cavities. He described the vascular connections between the periosteum and surrounding soft tissues and between the periosteum and the marrow. He concluded the latter was via the Haversian systems. Langer showed that the periosteal arteries arose from extraosseous branches of the nutrient artery and from arteries of the surrounding muscles, aponeuroses and fasciae particularly in the region of the interosseous membrane of the tibia. These arterial branches form encircling rings around the surface of the bone and are connected to each other by longitudinal vessels. He also showed that the periosteal arteries gave off branches that penetrated the cortex, the largest of these he found in the metaphyseal regions. He described in detail the vascularization of
cortical bone by periosteal arteries and veins as well as by the medullary circulatory system. He demonstrated the presence of multiple vessels in the Haversian canals and the frequent occurrence of large vascular spaces in the cortex. Finally, Langer also demonstrated that the epiphyseal arteries arose separately from capsular vessels. Langer's work was exceptional and many of his findings have subsequently been confirmed by other investigators.

Lexer and his associates (1904) extensively employed radiographic methods following the perfusion of radiopaque substances. He identified three vascular regions in long bones; diaphyseal, metaphyseal and epiphyseal and demonstrated that branches of the nutrient artery only exceptionally penetrated the growth plate. He showed that the largest venous drainage channels were in the metaphysis. Unfortunately, a lot of these earlier works lacked detailed information on the blood vessels once they entered deeply into bone. Many of the injection materials in common use at that time were either too viscous to penetrate vessels of small sizes or diffused excessively and blurred the limits of individual vessels. Consequently, most of the illustrations of the arrangement of the fine vessels in bones were based on assumptions.

More recently many workers, notably Barclay (1951), have used the technique of micro-arteriography in which radiopaque dyes are injected into the main arteries of a limb and then thin sections, for example 400μ thick, of the perfused bone are obtained and radiographed. Using this method they
outlined the fine vessel arrangements of the osseous circulation. Micropaque or aqueous barium sulphate suspension was commonly used to delineate the afferent pathways and dyes such as India Ink, Berlin Blue and Methylene Blue were used to fill capillary beds (Trueta 1968, Brookes 1971, Trias & Frey 1979). de Marneffe (1951), Wray and Lynch (1959) and Lopez-Curto et al. (1980) used plastic materials for vascular perfusions. Paraffin histology has been a useful auxiliary technique in establishing the nature of perfused vessels and their relationship to the bone (Kelly et al. 1959). Branemark (1959) pioneered the technique of vital microscopy which he used to examine the medullary vessels. The electron microscope has also been exploited in recent times to demonstrate the intricate three-dimensional branching and interconnection of cortical vessels (Farkas et al. 1984).

1.21 THE VASCULAR ANATOMY OF BONE

The techniques of intravascular perfusion, microradiography and accurate photographic reproduction have been instrumental in unravelling the basic arrangement of the intraosseous vasculature. The arrangement in man is believed to be similar to that elucidated in all the animals studied so far (Trueta 1968, Brookes 1971). The diaphysis would appear to receive blood from three different sources; namely, nutrient, periosteal and epiphyseo-metaphyseal respectively.

1.211 The principal nutrient artery (arteries)

In general, a systemic artery running parallel to the
longitudinal axis of a long bone gives rise to one, occasionally more, principal nutrient arteries which perforate the diaphysis (Langer 1876, Warwick & Williams 1980). The artery enters the marrow cavity through an oblique channel in the cortex called the nutrient foramen without giving any branches to the compactum (Morgan 1959, Nelson et al. 1960). It divides into two trunks, ascending and descending, shortly after emerging into the marrow cavity (Fig. 1a).

The ascending trunk initially points distally where it gives off a number of straight branches which traverse the marrow and pierce the cortex. The arterial trunk then turns abruptly upwards and divides into two or more ascending branches which pass directly upward towards the metaphysis surrounded on all sides by the sinusoids and cells of the marrow. The descending trunk passes down the marrow cavity towards the distal metaphysis and terminates in two or three branches which provide arterial twigs to the cortex and marrow of the metaphysis.

Cortical branches are derived successively from the arterial trunks and they run a characteristic course, first parallel to the main vessels then turning sharply back on themselves to form loops as they pass to their destinations. In the metaphysis the main vessels break up into vertical leashes of vessels which divide and subdivide into fine straight branches (Trueta 1968). Anastomotic connections link these terminal arteries with similar vessels derived from an epiphyseal–metaphyseal circulation. The nature of the
anastomotic connections is not clearly established and it is not known whether it occurs at capillary or arteriolar level.

1.212 The periosteal arteries

These arise from a systemic vessel at fairly regular intervals along the length of the bone (Langer 1876). They pass on to the periosteum accompanied by paired venae commitantes and are segmentally arranged in pairs which encircle the bone as rings. Anteriorly, they break up into branches which form complex woven anastomoses with adjacent rings. Many fine long anastomoses are present between the vessels of adjacent rings and between the periosteal vessels and the epiphyseo-metaphyseal vessels at the ends of the bone (Fig. 1b).

From this dense and intricate network of vessels arise branches which form capillary plexuses in the osteogenic layer of the periosteum lying next to the bone surface. Some vessels derived from the periosteal vascular network are incorporated into the diaphyseal cortex (Langer 1876, Zucman 1960, Simpson 1985). In areas of fleshy muscle attachments, the intramuscular and periosteal arteries appear to be in continuity (Zucman 1960) but, according to Brookes (1971), few periosteal branches of arteriolar size penetrate the cortex.

1.213 The epiphyseo-metaphyseal arteries

Yet other "nutrient" vessels are associated with the
metaphyseal ends of long bones (Fig. 1b) and micro-arteriography reveals that these arteries also ramify in the marrow (Trueta 1968, Brookes 1971). The epiphyseo-metaphyseal arteries enter the bone via a number of nutrient foramina and their stem vessels are derived from a vascular circle around the extremities (Hunter 1743). Morgan (1959) has pointed out the presence of anastomoses between the vessels constituting the vascular circle and the longitudinal periosteal arteries.

The epiphyseo-metaphyseal arteries are important for bone growth (Brookes 1957) and their overall capacity as reflected by their cross-sectional area is similar to that of the principal nutrient artery with which they communicate. They do not directly contribute to diaphyseal vascularization but are thought to provide blood to the peripheral zones of the metaphysis and adjoining diaphysis (Morgan 1959, Brookes 1971) presumably through longitudinally running intracortical vessels.

1.2.14 The venous drainage

Most accounts of the blood supply of bone omit detailed descriptions of the venous drainage because this aspect of the osseous vasculature has been difficult to demonstrate conclusively. Consequently, there is no clear agreement concerning the venous pathways but most workers now believe that the venous arrangements differ significantly from the arterial. The principal venous structure is usually described as a thin-walled central venous sinus, four to five times the
size of the nutrient artery (Fig. 2a), which apparently extends the full length of the diaphyseal marrow (Morgan 1959, Trueta & Cavadias 1964). Kelly (1968) and other authors have suggested that this structure is continuous with the nutrient veins but Morgan (1959) believes that it drains into the systemic veins via unnamed transcortical channels. Transverse vessels or sinusoids (Fig. 2b) apparently drain directly at regular intervals into this central venous sinus (Trueta 1968, Brookes 1971, Thomas et al. 1982, Thomas 1983). The metaphysis appears to be drained separately via large veins which accompany the arteries (Lexer et al. 1904), however, Tilling (1958) has demonstrated anastomotic connections between these and nutrient vessels.

Other workers have suggested additional features to this account of the venous drainage of bone. Harrison and Gossman (1955) proposed that some intraosseous channels contained small veins which emptied directly into the systemic veins. Brookes (1960) described the venous channels of bone in terms of a spray of vessels lying within the cortex. Cortical venous channels were also identified by Harrison (1966) who was able to produce simultaneous dilatation of the Haversian systems and the Volkman’s canals.

1.22 THE BLOOD SUPPLY TO THE DIAPHYSEAL CORTEX

There are three rival theories concerning how blood is supplied to the diaphyseal cortex. The account of the gross vascular anatomy of long bones given above would appear to suggest three possible and separate sources of blood supply.
to the diaphyseal cortex; namely, periosteal, nutrient (medullary) and epiphyeseo-metaphyseal (Lexer et al. 1904, Brookes 1971). However, this does not appear to be the case since only medullary vessels have been shown regularly by intra-arterial perfusion and micro-radiographic techniques to ramify significantly within the cortex. A rather exclusive medullary blood supply to the bone cortex is, therefore, widely favoured by many investigators (Macnab 1958, McAuley 1960, Rhinelander & Baragry 1962, Rhinelander 1968, 1972, 1974, Brookes 1971). Others notably Crock (1967) and Trueta (1960) do not accept this.

1.221 The nutrient artery blood supply concept

Many workers notably Brookes (1971) and Rhinelander (1968, 1972, 1974) have demonstrated that cortical nutrition is derived almost exclusively from the nutrient circulation (Fig. 3a). These authors assume that only vessels functional at the time of perfusion are filled and that the direction of blood flow is uniform and reflected in the vascular branching. They point out that the direction of blood flow through the cortex is entirely centrifugal (Fig. 3b).

The nutrient veins in the nutrient canal are generally smaller than their companion artery (Warwick & Williams 1980) and, therefore, much of the blood entering bone by way of the nutrient artery leaves by routes other than the nutrient veins. Brookes and his colleagues (1961) demonstrated direct evidence of centrifugal flow by intraosseous perfusion using micropaque. Ficat and Arlet (1980) proposed that
transcortical channels provided these additional routes. Brookes (1971, 1987) has reviewed the evidence in support of a centrifugal direction of blood flow through the diaphyseal cortex and, hence, the nutrient artery supply concept. Lamas et al. (1944) and more recently Gunst (1980) observed that a dye injected intravenously discoloured the cortex progressively from within outwards. Dillaman (1984) observed a concentration gradient across the diaphyseal cortex after systemic injection of ferritin which decreased from the endosteal to the periosteal surface.

In addition, after maturity the periosteum covering the diaphysis is reduced to a most tenuous structure indeed and, therefore, it is suggested that its importance with regards to the blood supply of the diaphyseal cortex reduces with age. Rhinelander (1974) has claimed that periosteal supply to the diaphyseal cortex under normal circumstances was significant only in the vicinity of heavy fascial attachments. For these reasons, the classical concept of a substantial contribution of periosteal blood to cortical nutrition (Fig. 4), which implies a centripetal flow of arterial blood into the cortex from overlying periosteal vessels (Langer 1876, Crock 1967, Trueta 1968), has been discredited.

However, the concept of a wholly medullary blood supply for the diaphyseal cortex appears to be at variance with certain known clinical facts. The most relevant is that in clinical practice viability does not appear to be a problem in the main proximal and distal fragments of a shaft fracture.
even where the nutrient arterial supply would be expected to have been interrupted. Furthermore, the technique of reaming and intramedullary nailing of fractures with close fitting nails, which presumably completely destroys the normal medullary circulation and most of its epiphysio-metaphysial connections as well as preventing medullary revascularization, does not appear to interfere significantly with normal healing (Trueta & Cavadias 1955, Kuntscher 1968).

Furthermore, it may be difficult to accept the view often expressed by the protagonists of the exclusive medullary blood supply concept that the stripping of the periosteum and/or the application of a plate causes bone necrosis only because the efferent outflow of blood from the marrow is obstructed rather than because the periosteal arterial supply has been destroyed (Rhinelander 1968, 1972, 1974, Brookes 1971). It may be argued that cortical venous obstruction by these means is not likely to be significant in clinical practice because the area of the cortex stripped of its periosteum or occupied by the plate is usually only a small fraction of the entire periosteal surface available for drainage. There is no evidence to suggest that these types of venous obstructions could not be effectively bypassed (Cuthbertson et al. 1965) by longitudinally running intracortical vessels or that venous connections do not rapidly re-establish after fracture.

It has been a consistent finding by several workers, in particular Drinker et al. (1922) and Huggins and Weige (1939), that there is extensive communication between the
three osseous circulations. Several workers including Brookes (1971) have pointed out that there must exist direct vascular continuity between periosteum and cortex and between cortex and marrow. Weiland and his colleagues (1982), who studied cortical perfusion by the hydrogen wash-out technique in partially osteotomised canine ribs, demonstrated normal cortical perfusion in these bones that have had their medullary circulations deliberately interrupted. However, the study by Weiland et al. (1982) was not an anatomical study and, therefore, they did not demonstrate the sources of continued blood supply to the diaphyseal cortex deprived of medullary supply.

The concept of an exclusive medullary blood supply relies principally on the evidence from arterial perfusion. However, in interpreting the results of arterial perfusion, it is often overlooked that there are many reasons why a vessel may not be demonstrated. Perfusion techniques depend upon overcoming the resistance of vessels and are, therefore, biased against high resistance vessels. Periosteal arteries are generally of finer calibre than the nutrient arteries and consequently, may be of higher resistance. If periosteal arteries are to contribute to the nutrition of the cortex they may need to be of a higher resistance in order to overcome the intramedullary pressure which some believe is high (Shaw 1964, Brookes 1971). Thus, the high resistance of periosteal arterioles could account for the inability to demonstrate their presence in the normal diaphyseal cortex using simple perfusion techniques.
The dual or periosteal-nutrient blood supply concept

The various observations which cast doubt on an exclusive nutrient blood supply to the cortex have prompted some workers, notably Crock (1967) who carried out vascular perfusion studies in human lower limbs after death, to revive the concept of a dual blood supply (Fig. 4a). Under this scheme, the cortex receives direct arterial input simultaneously from periosteal as well as from nutrient sources. Albinus (1756) and Crock (1967) using post-mortem arterial perfusion have demonstrated periosteal arterioles which they claim traverse the cortex to supply the marrow. Langer (1876), Trueta (1968) and more recently Trias and Frey (1979) using Indian ink injections, were able to show that periosteal arterioles penetrated the cortex.

A bi-directional blood flow was envisaged by this concept (Fig. 4b) and Trueta (1968) has demonstrated experimentally the circumstances in which centripetal blood flow through the cortex could occur. However, this view has been challenged by Brookes (1971) who pointed out that it implied that the periosteal and the nutrient arterial currents meet head on. This, Brookes claimed, would lead to a circulatory standstill in the cortex but this argument may not be entirely tenable. As McPherson and Shaw (1961) have pointed out, there is no reason why centripetal flow cannot occur in one set of capillaries simultaneously with centrifugal flow in another set of capillaries, a situation which occurs in the heart. On the other hand, the portal circulation in the liver provides an example of a
circumstance where different currents of blood meet without affecting the overall direction of blood flow.

There are other reasons to suppose that periosteal arterioles could participate in the blood supply of the diaphyseal cortex. For instance, the periosteal vessels are vital to the growth of long bones by the mechanism of appositional growth in childhood. The new layers of bone added to the outside of the cortex are constructed around these vessels (Ham & Cormack 1979). Blood vessels from the periosteum become successively buried in each new layer of bone and, as successive layers of bone are buried, their vessels retain connections with the periosteal circulation (Trueta & Harrison 1953, Trueta 1963). Thus, the entire diaphysis appears to be constructed around the periosteal vessels and it is, therefore, inconceivable that these vessels would be excluded from supplying blood to the cortex.

Furthermore, according to several workers notably Drinker et al. (1922), Huggins and Weige (1939) and Crock (1967), it is because there are extensive intracortical anastomoses between the periosteal and the medullary circulations that damage to one could be made good by the other. Hence, one can strip the periosteum off a fractured bone with seeming impunity provided the nutrient vessels remain intact. Alternatively, one can ream completely and nail the medullary canal provided the periosteum remains intact without fear of causing a massive necrosis of the bone (Macnab 1958, Crock 1967).
Rhinelander’s (1972, 1974) functional afferent-efferent blood supply concept of the osseous circulation retains a strong commitment to the supremacy of the nutrient blood supply and attempts simultaneously to account for the paradox whereby the destruction of medullary blood supply was compatible with a normal cortical blood supply. According to this hypothesis, the periosteal arterioles have the potential to supply the cortex with blood particularly in areas of heavy fascial attachments to bone but this is a reserve function only called upon in times of emergency. Thus, the periosteal arterioles have a role only in pathological circumstances and, even in such circumstances, they are usually reinforced by an extraosseous blood supply.

Rhinelander (1972, 1974) envisaged that the arterial supply to bone was arranged as one unit or afferent system (Fig. 5a). This consists of a series of anastomoses between components of the principal nutrient artery, epiphyseal-metaphyseal arteries and periosteal arterioles. It is from this afferent network that blood is distributed to every part of the bone although the principal nutrient artery provided most of the blood. Lopez-Curto et al. (1980) have demonstrated the conduit vessels connecting the nutrient arteriolar system with periosteal vessels (Fig. 5b).

The afferent network is complemented by an efferent arm which also forms a functional unit. This apparently consists of the large emissary veins, venae comitantes of the
principal nutrient artery, cortical venous channels and periosteal venules. The presence of a central venous sinus is denied by the protagonists of this concept presumably because its acceptance negates the mirror image relationship between the afferent system on the one hand and the efferent system on the other.

This concept of the blood supply of the diaphyseal cortex, which is based entirely on perfusion studies after fracture, attempts rather too passionately to downgrade the role of the periosteal circulation in fracture healing. Consequently, even the increase in the numbers of periosteal arterioles observed by Rhinelander after fracture was ascribed entirely to a newly developed extra-osseous blood supply while a similar increase in the numbers of medullary arterioles was not so regarded. Although the functional blood supply concept does proffer an explanation for the apparent ability of cortical blood to reverse flow almost instantaneously from centrifugal to centripetal when called upon to do so, it seems to the author inconceivable that a blood supply derived solely through heavy fascial attachments could maintain cortical viability of fracture fragments.

1.224 The arrangement of vessels within the diaphyseal cortex

The detailed arrangement of vessels within the cortex is not known with any certainty and, therefore, continues to be a source of considerable controversy. Available information is scattered and contradictory and often there is no differentiation in many reports between the various
components; arterial, venous or capillary. The different concepts of the blood supply of bone outlined above do not directly address this problem.

Many workers notably Ham and Cormack (1979) have proposed that cortical vessels are longitudinally orientated within the Haversian system and that these anastomose with one another through horizontal short limbs lying in the Volkmann's canals. By contrast, de Narneffe (1951) and Cohen and Harris (1958) found that typically cortical capillaries are oblique in disposition and Brookes (1971) has shown that cortical vessels radiate fan-shaped from mid-diaphysis towards either metaphysis. Recently, Albrektsson (1985) has described apparent differences between the cortical capillary network of the young which he claims is longitudinal and the adult where it is more transverse and anastomosing.

Furthermore, Cohen and Harris (1958) suggested that the Haversian canals increase in size within the bone substance with the larger vessels near to the endosteum and the finer ones near to the periosteum. This is compatible with centripetal flow where cortical capillaries would progressively widen to form venules as they approached the endosteal surface. However, Brookes (1971) in his classic treatise on this subject has pointed out that some of the endosteal vessels contain a coat of pericytes and are, therefore, technically arterial branches to the cortex. Arterial perfusion studies have been interpreted as indicating that the vessels are on the arterial side of the circulation with terminal medullary arterioles as the parent
vessels. No equivalent venous perfusion has been carried out, however, to see whether these vessels could be filled retrogradely. Therefore, it is not known for certain to which side of the circulation, pre- or post-capillary, the cortical vessels belong.

In addition, Lopez-Curto et al. (1980) have revised the classical view which proposed that the medullary arterioles supplying the marrow sinusoids were in series with those supplying the diaphyseal cortex (De Bruyn et al. 1970). Instead they suggested two separate arteriolar systems derived from the nutrient artery; one to exclusively supply the marrow and the other the cortex. The cortical arteriolar system is thought to drain exclusively into the periosteal venous network. The report by Lopez-Curto et al. (1980) needs to be confirmed by others because it introduces a novel concept of bone circulation and because it is extensively quoted in the English literature. Clearly a lot of work still requires to be done to reconcile the various differences of opinion regarding the microvascular anatomy of the diaphysis of long bones.

1.23 THE PHYSIOLOGICAL RELATIONSHIPS BETWEEN THE OSSEOUS CIRCULATORY SYSTEMS

The relative contribution of each of the three arterial systems of bone to diaphyseal nutrition is very difficult to investigate. Nevertheless, an attempt to investigate the relative importance of each osseous circulation, as regards tissue viability and repair of defects or fractures, has been
made by selective suppression of one or other of these
circulations. Drinker et al. (1922) found a normal pattern
of cortical perfusion, as demonstrated by injected dye, in
the tibia of dogs after the soft tissues had been stripped
from the periosteum. A similar perfusion pattern was also
observed when the abdominal aorta was perfused after ligating
the nutrient artery. They concluded that the main feature of
the bone circulation was its extensive intercommunications.

1.231 The classical vascular isolation techniques

Johnson (1927) improved the method for research into the
blood supply of bone by interfering with two of the three
sources in order to completely isolate one or other vascular
supply to canine tibiae. To isolate the nutrient artery
supply, the periosteum was stripped from the shaft which was
then rubbed with bone wax. In addition, the medullary cavity
was blocked off with wax plugs at the metaphyses to cut off
epiphysio-metaphyseal anastomoses. To study the
epiphysio-metaphyseal circulation, the periosteum was
stripped from the shaft and the shaft was rubbed with bone
wax, and in addition, the nutrient artery was ruptured. To
isolate the periosteal blood supply, the nutrient artery was
ruptured and the medullary canal was blocked with wax plugs
inserted through drill holes at each end of the diaphysis.
Three cortical defects were created in the cortex of the
shaft after each isolation procedure to investigate repair
processes under each experimental condition. The animals were
perfused with India Ink and killed at intervals after
operation and the bones were examined histologically. He
concluded from these experiments that the nutrient artery was the most important source of cortical and medullary nutrition since it alone by itself could maintain the viability of the entire shaft and repair bone defects. He suggested that the periosteal supply was the least important but this conclusion is questionable because the residual blood supply was not investigated immediately after the vascular occlusion procedure. Therefore, it is not known, but has been assumed, which circulation has been isolated. Furthermore, simultaneous suppression of nutrient and metaphyseal circulations did not produce uniform results.

In the Johnson models, the shaft could not have been totally deprived of periosteal blood by periosteal stripping because some areas of the periosteum were left intact posteriorly to prevent damage to the nutrient artery and superiorly to prevent damage to the epiphyseal-metaphyseal vessels. These posterior periosteal strips belong to the areas of the periosteum particularly invested with muscle and fascial attachments from which the periosteal contribution to cortical nutrition is believed to be derived (Rhinelander 1972, 1974). Thus, in the medullary circulation only experiment, isolation is incomplete and, therefore, the model could over-emphasize the territory supplied by the nutrient artery. By contrast, as admitted by Johnson himself, nutrient artery ligation as described involved dissection which could have simultaneously damaged the periosteal supply to the shaft. Therefore, periosteal blood supply was probably also significantly damaged in the model with nutrient damage only. Furthermore, the periosteum could hardly have escaped being
traumatised as the five cortical drill holes for medullary plugging and for observing repair were being made. In addition, bone wax, which was used to prevent re-vascularisation, often disintegrated and did not adhere to the cortical surfaces. Thus, extraosseous contribution to cortical nutrition was never totally excluded in experiments preserving nutrient or metaphyseal circulation alone. Thus, in the periosteal circulation only experiment, part of this circulation is damaged and, therefore, the model could underestimate the territory capable of being supplied by the periosteal circulation.

Foster et al. (1951) investigated the problem again in femora of young and adult rabbits. Using Johnson's (1927) technique they found extensive bone and marrow necrosis after dividing the nutrient artery and stripping the whole periosteum from the shaft. The removal and replacement of the dead bone took 3 months in young rabbits and over 9 months in the adults. Cortical changes were limited to the inner parts of the cortex when the nutrient artery alone was divided. They concluded that the outer cortex must be supplied independently of the nutrient artery. These results suffer from the same limitations as Johnson's original work because the authors did not investigate the residual blood supply following each attempt at interrupting the osseous circulation. Consequently, it is not known how complete vascular isolation was in each case.
1.232 The modern vascular isolation techniques

Trueta and Cavadias (1964) investigated this problem in the radius of young and old rabbits but modified Johnson's (1927) original technique. They interrupted periosteal blood supply to a 2 to 2.5 cm segment of the diaphysis by subperiosteal or extraperiosteal implantation of polythene sheaths. The nutrient artery was interrupted by ligation at the nutrient canal and a polythene plug was inserted into the canal to obliterate it. The epiphyseo-metaphyseal circulation was interrupted by inserting polythene strips into drill holes in each metaphysis. The authors correlated normal radiographic appearances at intervals of time with micropaque-Berlin Blue micro-angiography and with histology.

Trueta and Cavadias (1964) found that when the periosteal circulation alone was left "undisturbed", new bone formation accompanied necrosis of half to two-thirds of the inner parts of the cortex in both young and old animals. The nutrient artery was not perfused although dye could be detected in the medullary cavity. The number of periosteal vessels penetrating the cortex were apparently increased.

By contrast, when diaphyseal nutrition was entrusted to the nutrient artery, there was no appositional growth. The nutrient artery and its branches were perfused and bone necrosis was restricted to the outer third of the cortex. Appositional growth was also prevented when diaphyseal nutrition was entrusted to the metaphyseal circulation alone. In addition, the nutrient artery was perfused in adult
animals and the marrow appeared normal although necrosis was still observed in the inner third of the cortex. In young animals, however, the nutrient vessels were not filled and there was total necrosis of the cortex and marrow of the middle section of the diaphysis.

These latter findings would appear to suggest that it is only in adult long bones that important arteriolar connections exist between the metaphysis and the marrow and between the metaphysis and the outer portions of the cortex. By contrast, in the immature long bones there is no vascular communication between the epiphysis and the metaphysis/diaphysis. Trueta and Cavadas (1964) also concluded from these results that the nutrient artery supplied exclusively the diaphyseal marrow and the inner two-thirds to three-quarters of the cortex. They further suggested that simple ligation of the nutrient artery without additional marrow damage was not effective in producing cortical necrosis, as has been observed by others including Huggins & Weige (1939), Foster et al. (1951), Cuthbertson et al. (1965) and Shim et al. (1968), because of alternative blood supply provided by periosteal vessels in the young and by epiphyseo-metaphyseal vessels in adulthood.

However, the findings of this important work are invalidated because Trueta and Cavadas (1964), like most of the earlier workers, did not seek to determine the residual vascularity in their various experimental models before any physiological adjustments occurred. This can only be achieved by conducting these investigations after death.
Furthermore, the materials introduced into the marrow cavity in the vascular occlusion studies described above often provoked considerable inflammatory response (Johnson 1927, Trueta & Cavadias 1964). There is no reason to suppose that this is not responsible for the changes observed in these experiments. In the "callus without fracture" experiments of Kuntscher (1968), cortical necrosis and new bone formation followed the introduction of rusty needles and vegetable oils into the medullary cavity. Kuntscher rightly observed that the phenomenon was due to the inflammatory processes induced by the implants. The probable mechanism is subperiosteal collection of the inflammatory exudate produced in the marrow cavity. As a consequence, the periosteum is elevated from the shaft and this leads to the interruption of the blood supply to the cortex.

On the other hand, Dankwardt-Lilliestrom and his colleagues (1969, 1970) have shown that cortical necrosis following intramedullary reaming may be due to secondary embolisation of cortical channels by and subperiosteal collection of marrow debris. This outcome can be prevented if an outlet is provided for medullary debris, for example, by multiple osteotomies in the shaft. Thus, vascular isolation techniques require substantial modifications to make their results acceptable.

Anatomical studies emphasize important extrasosseous connections between the three osseous circulatory systems of bone via the circulus arteriosus (Hunter 1743) and via
extraosseous branches of the principal nutrient artery (Langer 1876, Simpson 1985). There are intracortical connections via the Haversian systems and Volkmann's canals (Cohen & Harris 1958, Brookes 1971). Consequently, the relative contribution of the different osseous circulatory systems cannot be determined accurately without isolating a diaphyseal segment as a "free body" supplied by only one of the three possible sources in any given study. This experimental model, which has not been previously used to investigate the blood supply of the diaphyseal cortex, has been utilised in the present study.

1.24 THE BLOOD SUPPLY TO HEALING FRACTURES

The blood supply plays a prominent role in fracture healing. Consequently, vascular neogenesis is observed in abundance in histological sections of the fracture callus (Teneff 1950). In addition, important circulatory changes have been observed during fracture healing using perfusion techniques (Gothman 1961, Koekenberg 1963, Rhinelander 1968, 1972, 1974, Trueta 1968). However, there is little agreement at present on the relative importance of each of the potential sources of blood to the healing fracture.

The role of blood vessels in the healing of fractures has been the subject of debate since Haller (1763) opposed Duhamel's (1739) theory of periosteal bone formation with his vascular theory. Later Leriche and Policard (1928) provided the focus of opposition and there is still an unresolved controversy as to which blood supply, nutrient or periosteal,
plays the more important role in fracture healing. While some are dismissive of the periosteal contribution (Rhinelander 1968, 1972, 1974), others point to the fact that non union is inevitable if the periosteum is not preserved during open reduction of fractures (Crock 1967, Trueta 1968). Nelson et al. (1960), Brookes (1971) and Rhinelander (1972, 1974) have suggested a compromise that although periosteal blood supply plays a relatively minor role in supplying the normal adult diaphyseal cortex, its contribution increases after injuries that disrupt the medullary circulation. On the other hand, Macnab (1958) and Trueta (1963) have emphasized the prominence of the periosteal circulation during healing.

1.241 The concept of periosteal blood supply to healing fractures

The early investigators in general favoured a periosteal blood supply rather than a medullary supply in fracture healing. Kolodny (1923) who studied osteotomies of the radius of dogs confirmed the "fracture hyperaemia" described by Delkeskamp in 1906 (Trueta 1974) and by Lexer in 1922. He emphasized the importance of the periosteal vessels and concluded that an adequate periosteal supply was essential for normal union. He stressed that periosteal callus was more important than endosteal callus for fracture union since destruction of the nutrient artery did not affect callus formation unless periosteal vessels had also been destroyed. Macnab (1958) studied the blood supply of the shaft of the tibia and asserted that a fracture could heal without endosteal circulation but never without periosteal
circulation.

Gothman (1961) in a series of elegant experiments using rabbits and monkeys demonstrated the importance of the surrounding muscles in providing new vessels to replace the damaged blood supply. Wray (1963) has shown that the periosteal arteries augmented by vessels from the surrounding soft tissues were the main source of vascularisation of the fracture callus. Holden (1972) also emphasized the importance of the blood vessels derived from extraosseous sources. He demonstrated that the re-vascularisation of displaced segmental fractures of the rabbit radius occurred long before the re-establishment of continuity of the marrow vessels. He showed that re-vascularisation was delayed if the surrounding tissues were first rendered ischaemic.

1.242 The concept of medullary blood supply to healing fractures

By contrast, Ladanyi and Hidvegi (1954) found that medullary re-vascularisation was consistently richer than periosteal in healing osteotomies of the canine ulna. They compared simple external splintage with percutaneous wire transfixation and concluded that the repair of periosteal vessels was more rapid than that of the medullary vessels only when an implant was used to immobilise the fracture.

Rhinelander and his colleagues (1962, 1968, 1972, 1974) have extensively studied blood supply to healing fractures using micro-angiographic methods similar to Barclay's (1951).
They claimed that periosteal vessels played a significant role only in the vicinity of heavy fascial attachments and that under these circumstances a distinct, new extraosseous supply is developed. They differentiated different vascular patterns in response to displaced or undisplaced fractures. In displaced fractures, a periosteal network derived from torn muscles in the vicinity of the fracture predominated initially but this was only temporary. This they suggested was because fracture haematoma which may be extensive initially impeded the advance of proliferating medullary vessels. In undisplaced fractures, medullary vessels played the major role from the outset since not all vascular connections were severed. Similar views have been expressed by Johnson (1927) and by Brookes (1971) who suggested that the importance of a particular anatomical route to the fracture site depended upon the healing requirement.

1.243 Anatomical studies of the blood supply to healing fractures

The reports by Rhinelander and his colleagues (1962, 1960, 1972, 1974) require further comment as they are frequently quoted by other workers. They created fractures in dog tibiae by means of an hydraulic press which would have caused significant crush injury to the peristeme and the surrounding soft tissues. The fracture was stabilised by external plating which would have traumatised further the normal periosteal supply. Little wonder that they found a predominance of medullary circulation during the healing of their fracture model. It is instructive to note that when
this team created fractures of the radius by similar methods but manipulated the fracture to ensure significant displacement the blood supply was provided from both periosteal and medullary vessels. It is particularly relevant to note that no injection studies were carried out immediately after fracture to determine what damage had been caused to each circulatory system by their fracture technique. In addition, the authors admit to selective presentation of data in a manner calculated to emphasize a particular point of view. The authors made no serious attempt to explain why their results were not consistent from one animal to another within each of their experimental groupings. These shortcomings undermine the value of this work and any references to it by other workers should of necessity be viewed with caution.

Certain conclusions are inevitable if one accepts that the cortex is exclusively supplied by the nutrient artery as proposed by Rhinelander (1968), Trueta (1968), Brookes (1971) and many others. Theoretically, a fracture of the shaft of the tibia occurring after the nutrient artery has divided into its ascending and descending trunks should result in complete ischaemia of the bone beyond (Compere 1949, Jackson & Macnab 1959), unless open anastomoses exist between periosteal, medullary and epiphyseo-metaphyseal circulations but this has not actually been demonstrated experimentally. Thus, healing time could depend on the length of the fragment not receiving direct medullary blood supply (Watson Jones 1943) since the affected fracture fragment would need to be re-vascularised before it took part in the healing process. This is not
observed in clinical practice where the location of fracture in the shaft has not been found to influence the speed of healing (Ellis 1958a, Nicoll 1964, Johner & Wruhs 1983).

Furthermore, intramedullary nailing particularly of fresh fractures should lead to complete sequestration of the nailed bone but this state of affairs does not occur in practice. Experimental support for this has been provided by many workers notably Weiland et al. (1982) who demonstrated that cortical blood flow is not disturbed immediately after blocking the flow of blood through the marrow.

1.244 Vascular isolation techniques in the study of the blood supply to healing fractures

Progress in the understanding of the part played by blood vessels in the repair of fractures has been increased by the use of selective vascular suppression such as those described earlier. Trueta and Cavadas (1955) and Cavadas and Trueta (1965) used these techniques to study fractures of the rabbit radius. Like Ham (1930), they reported that in normal healing both periosteal and endosteal fracture callus proceeded from either side of the fracture. Endosteal callus appeared at the same time and did not precede the periosteal callus which was more extensive and more elongated (Ham 1930, Pritchard and Ruzsika 1950, Ham & Harris 1971, Ham & Cormack 1979). Initially, the new periosteal vessels pointed radially to the fracture line but did not reach it. Continuity of the nutrient vessels was re-established by proliferation from the damaged ends (Gothman 1961).
When periosteal circulation alone was the source of blood to the fracture site, the appearance of callus was not delayed nor was its shape altered. Bony union occurred within the normal 2 to 3 week period. Endosteal callus failed to appear, the nutrient artery and its branches were not perfused by injected dye and there was necrosis of the inner half of the cortex. By contrast, when the nutrient artery was the only source of blood there was complete ischaemia in a large number of experiments. This outcome was inexplicably blamed on the possibility of inadvertent damage to the nutrient vessels during operative manoeuvres. In the few animals without total cortical ischaemia, subperiosteal implantation of a polythene sheath around the fracture site led to the development of new layers of bone covering the implant. Periosteal callus formation was not reported with extraperiosteal implantation, rather an endosteal callus plug developed at about 2 weeks spreading from within outwards to effect union in 5 to 9 weeks. A number of these animals developed non union. Arterial perfusion studies revealed dye in the nutrient artery and its branches. Similar results were obtained when metaphyseal circulation provided the only source of blood supply but the incidence of delayed union and non union was higher still. It was concluded from these results that the contribution of periosteal vessels to the organisation of fracture callus was much greater than that of the nutrient vessels.

Inexplicably, these studies by Trueta and his colleagues (1955, 1965) have received less recognition in the English
literature than many poorly designed studies. Conclusions on
the blood supply to the fracture callus based solely on
experience with and/or experiments using intramedullary
nails, plate fixation or external fixators are commonly cited
but these results are particularly misleading. What has been
inferred is often presented as fact and very little attempt
is made in most studies to define in precise terms the
initial vascular injury sustained. The classic work by Trueta
and his colleagues (1955, 1965) needs to be repeated with
this in mind. However, stringent attempts would need to be
made to ensure that a suppressed circulation was totally
suppressed. An attempt has been made in this present study
to refine the Trueta model to take account of these
deficiencies.
Figure 1a The principal nutrient artery and the periosteal arteries
circulus vasculosus articuli
metaphyseal artery
epiphyseal artery
systemic artery
medullary artery
periosteal artery

Figure 1b The epiphyseo-metaphyseal arteries
Figure 2a Diagram of the venous drainage of bone proposed by de Marneffe (1951)
Figure 2b  Diagram of the medullary transverse veins (sinusoids) after Thomas (1983)
Afferents | Vascular Lattice | Efferents
---|---|---
epiphyseo-metaphyseal arteries | medullary sinusoids | collecting sinuses: epiphyseo-metaphyseal veins; nutrient and emissary veins
nutrient artery | cortical sinusoids | interfascicular venules
periosteal arteries | periosteal capillaries | intramuscular veins

Figure 3a  The medullary (nutrient artery) blood supply concept (Brookes 1971)

Figure 3b  Diagram of the vascular arrangements in the diaphyseal cortex proposed by Brookes (1986)
Afférents | Vascular Lattice | Efferents
---|---|---
epiphyseo-metaphyseal arteries | medullary sinusoids | collecting sinuses: epiphyseo-metaphyseal veins; nutrient and emissary veins
periosteoal arteries | cortical sinusoids | interfascicular venules
nutrient artery | | intramuscular veins
periosteal veins

Figure 4a. The dual blood supply concept (Crock 1967)

Figure 4b. Diagram of the vascular arrangements in the diaphyseal cortex proposed by Testut and Latarjet (1948)
Afferents: epiphyseo-metaphyseal arteries, principal nutrient artery, periosteal arteries

Vascular Lattice: medullary sinusoids, cortical sinusoids

Efferents: collecting sinuses, epiphyseo-metaphyseal veins, nutrient and emissary veins, periosteal venules, periosteal capillaries

Figure 5a The functional blood supply concept (Rhinelander 1974)

Diagram of the vascular arrangements in the diaphyseal cortex proposed by Lopez-Curto et al. (1980)

P.V. Periosteal vein
E.V. Emissary vein
SIN Sinusoids
N.V. Nutrient vessels
CAP Capillaries
C.M.S. Central medullary sinus

Figure 5b
1.3 FRACTURE HEALING

A fracture begins to heal as soon as the bone is broken and the events leading to the healing of a fractured bone are thought to be essentially similar to those which occur in any wound. They comprise the removal of damaged tissue and its replacement with a new tissue. However, bone is the only connective tissue capable of regeneration whereby the new tissue produced is identical to that which was damaged. Unlike other connective tissues, bone is not repaired with a scar.

1.31 NORMAL HEALING

Although the healing process proceeds through a number of recognisable stages, there are differences of opinion concerning certain aspects. The histological features at various time intervals after injury have been well described by Ham (1930) but the sequence of events is the subject of some dispute.

1.311 The haematoma concept of fracture repair

Traditionally, the repair of bone was envisaged to involve a series of calluses (Gallie & Robertson 1920, Leriche & Policard 1928) which replace each other after the original fracture haematoma has been transformed into the first callus (Fig. 6a). The haematoma is formed in the space
between the fractured bone ends, in the soft tissue spaces around the fracture site and in the medullary canal as a consequence of disruption of vessels. The haematoma is invaded by granulation tissue (Potts 1933) as elsewhere in the body. Macrophages remove the haematoma at the apices of the capillary loops of which the granulation tissue is composed and simultaneously, pluripotent mesenchymal cells (Young 1962, Trueta 1963, Owen 1970) lay down a loose connective tissue. This connective tissue is composed of collagen fibres embedded in a gel of proteoglycans and this subsequently becomes calcified over a period of weeks.

According to this hypothesis, the fracture callus is distributed throughout the area originally occupied by the fracture haematoma. The size and shape of the callus are determined by the haematoma. The majority of cells involved are brought by invading blood vessels to the fracture site (Murray & Becker 1970, Trueta 1963) and the only active cell division is found in the vanguard of the granulation tissue invasion.

1.3.12 The periosteal concept of fracture repair

By contrast, Ham (1930) has questioned the participation of a fracture haematoma in bone repair. He denied the existence of a fracture haematoma in the sense of a circumscribed tumourous mass in most fractures and asserted that when present, it provided physical impediment to healing. Sevitt (1981) expressed similar views in his treatise on fracture healing. Fracture repair is envisaged by
this concept as a deliberate and purposeful cellular phenomenon designed primarily to restore the mechanical integrity of the fractured bone (Charnley 1970). Consequently, healing proceeds in an orderly manner from one fracture fragment to another (Fig. 6b) and only one callus is formed.

According to this hypothesis, the osteoprogenitor (Young 1962) or bone-forming stem cells of the periosteum and endosteum close to the fracture site are stimulated to proliferate within hours of injury. This has been confirmed by autoradiography (Tonna & Cronkite 1961, 1962). Over the next few days the periosteum and endosteum become several layers thick and in time a distinct collar of cells is formed around each fragment some distance away from the fracture site. The two encircling callus collars then grow or migrate progressively towards each other eventually to meet and fuse. This cellular response to injury is referred to by McKibbin (1978) as "primary callus response". The repair tissue formed at this stage may be relatively avascular (Duthie & Barker 1955) because, as the osteoprogenitor cells proliferate so do the capillaries amongst them, but the latter do not grow as quickly as the cells (Girgis & Pritchard 1958).

In addition to proliferating, the osteoprogenitor cells also differentiate. The cells differentiating in the presence of abundant blood supply, which according to Ham (1930) is close to the shaft, become osteoblasts and those farthest away differentiate into chondroblasts. This apparently accounts for the presence of fibrocartilaginous tissue in the
fracture callus in the early stages of repair (Girgis & Pritchard 1958, Ham & Harris 1971). The cartilage developing in a callus has a temporary existence only, however, and is eventually converted to bone by the process of endochondral ossification (Ketenjian et al. 1978).

1.313 The competing blastemata concept of fracture repair

A third group believes that fracture repair is effected both by the stimulation of cells with existing osteogenic power and by inducing that power in other tissues (Fig. 6c). McKibbin has discussed this concept at length in his classic review of 1978. After fracture, these two processes apparently combine to form one osteogenic blastema (Pritchard 1963) from which the new bone is derived. The osteogenic blastema is centrally placed from where it invades the fracture haematoma. This is believed to be separate from a fibrous blastema, which is derived entirely from the surrounding soft tissues. The fibrous blastema is peripheral and normally serves to restore the continuity of the fibrous periosteum.

Fracture repair is regarded as a competition between the two blastemata (Hult 1980, 1981) and where the fibrous elements invade the fracture site before the osteogenic blastema, union may be prevented. However, incorporated into this concept is the belief that under certain circumstances, the fibrous elements invading a fracture site may be transformed into bone (Mulholland & Pritchard 1959, Sevitt 1981).
1.314 Sources of osteogenic cells for fracture repair

The differences of opinion concerning the sequence of events in the healing of fractures arise in part from differing concepts of the sources of osteogenic cells for fracture repair. All agree that the actual laying down of bone is the function of osteoblasts, the dispute is about their origin and the factors which stimulate their activity. There are two divergent views and a compromise concept.

According to one view, bone repair is effected by specific cells belonging to the bony tissue and resident in its membrane coverings; viz, periosteum and endosteum (Enneking 1948), and in the tissues of the Haversian canals. This concept developed from the work of eighteenth century researchers such as Duhamel who identified the periosteum as the origin of the fracture callus by feeding red madder to animals and Ollier who demonstrated that cells in the periosteum produced bone (Keith 1919). Young (1962) called these cells the osteoprogenitor cells and proposed that they have a predetermined commitment to bone formation. Central to this concept is the argument that fracture healing amounts to parenchymal repair since a scar is not formed. In similar circumstances elsewhere in the body, for example, as in liver regeneration, repair is usually effected by stem cells resident within the injured tissue or organ.

In the opposing view, some form of metaplasia is assumed whereby non specific cells of young connective
tissues differentiate into bone-forming tissues. Evidence has been accumulated to the effect that bone marrow reticular cells (Denis 1957, Burwell 1964), vascular endothelial cells (Trueta 1963) and "totipotent" mesenchymal cells (Young 1962, Owen 1970) could be induced to form bone. The phenomenon which is called osteoinduction (Urist 1965) has been used to account for bone formation at ectopic sites. This concept which was proposed in the eighteenth century became more established after Macewen (1912) had denied that the periosteum was osteogenic.

Some authors accept both theories (McKibbin 1978). They believe that the sources from which osteogenic cells are derived in the repair of a particular fracture depends upon a number of factors. For instance, with rigid immobilization an external callus is not formed instead a direct fragment to fragment repair is effected by the tissues in the Haversian canals (Schenk & Willenegger 1967). On the other hand, where a significant fracture gap is present periosteal callus alone may not be sufficient to bridge the gap, consequently, union is achieved through mesenchymal proliferation within the gap (Pritchard 1961).

Nevertheless, whatever the views about the probable sources of osteogenic cells for the repair of fractures, no convincing morphological sequence of events other than that of Ham (1930) has been proposed. On the other hand, the biological importance of the ability of mesenchymal cells to be induced to form bone is not known. Furthermore, their role in fracture healing is entirely speculative.
1.315 The stimulus for osteogenesis in normal fracture repair

The concept of multiple origins of osteoblasts introduces a very broad view of cellular differentiation which allows cells of mesenchymal origin to transform from one type to another. The stimulus for "recruitment" to bone formation specifically in the circumstances of healing fractures is thought to be a chemical one derived from necrotic bone cells in the region of the fracture (Urist & McLean 1952, Trueta 1963). Bridges & Pritchard (1958) suggested that it may be a protein and Trueta (1963) proposed that it was a vascular stimulating factor (VSF). The evidence cited in support of a humoral stimulus includes a generalised increase in bone formation throughout the skeleton observed in rats following closed tibial shaft fractures (Wray & Schneider 1969).

Most orthopaedic surgeons will accept that the presence of bone, whether viable or nonviable, is a major stimulus to osteogenesis. A bone graft laid alongside a fibrous union between fracture fragments is subsequently able to effect bony union. According to Urist (1965), bone possesses a specific diffusible substance, bone morphogenetic protein (BMP), which is liberated in the vicinity of a fracture. This substance has been shown by several workers (Drivdahl & Howard 1982, Sato & Urist 1984) to be capable of inducing primitive mesenchymal cells to form bone but its role in fracture healing has not been proved.
Other workers propose that fracture healing is stimulated by a change in the local mechanical environment. It is for this reason that it is argued that rigid immobilization produces healing by primary intention (Schenk & Willenegger 1967) while functional bracing encourages copious proliferative repair (Sarmiento et al. 1977). The presence of piezoelectrical and other bioelectrical potentials in bone (Yasuda 1954) has now provided the biological explanation for this phenomenon. Bassett (1962) demonstrated bone formation and bone destruction in association with electonegative and electropositive potentials respectively. Friedenberg and Brighton (1966) showed that changes occur in the intrinsic biopotentials of bone following fracture and that these changes provide the stimulus for new bone formation. However, electrical stimulation of osteogenesis is not nearly as successful in clinical practice as it is in the laboratory.

Further, although mechanical factors such as motion at a fracture site (Friedenberg & French 1952, Yamagishi et al. 1955, Sarmiento et al. 1977) are known to influence the size of the callus formed at the fracture site, there is little hard evidence to support the view that mechanical factors per se initiate osteogenesis during fracture healing. Motion at the fracture site probably increases callus size through repeated fracturing of the callus such that each fracture episode is regarded as a fresh fracture by the osteogenic cells. On the other hand, the fact that the external callus is abolished by rigid immobilization may be interpreted as suggesting that the callus size depends upon how quickly the
"continuity" of the fractured bone is restored (Charnley 1970, Ham & Harris 1971).

In addition, there is some clinical evidence that rigidly immobilised fractures do not heal by primary bone union since if a fracture so immobilised unites there is usually some evidence of periosteal callus. It is likely, therefore, that a periosteal factor is a major component of the signal for fracture repair. Furthermore, although motion at the fracture site increases the size of callus formed, there appears to be a limit to its size. This raises the possibility that callus size may depend on growth limiting factors such as availability of nutrition, and therefore, the blood supply.

1.32 DELAYED UNION AND NON UNION

In clinical practice, fracture union is difficult to define in precise terms. A fracture is said to be clinically united when there is no local tenderness or movement at the fracture site and when non-protected weight-bearing can be commenced. However, the signs of clinical union may be present when continuity at the fracture site has been achieved by fibro-cartilaginous tissue and not by bone (Ham et al. 1938, Ham & Harris 1971). Clinical union precedes radiographic union and may be observed as early as six weeks after fracture (Watson Jones & Coltart 1943) while radiological union strictly defined as visible bridging callus and trabecular continuity between fracture fragments may take several more weeks. The point at which a particular
fracture is regarded as united depends to a large extent on the observer.

In practice a fracture with bridging callus surrounding more than 50% of the diaphyseal circumference at the fracture site, with no pain or motion on clinical stressing may be regarded as united. The average time taken by a particular type of fracture to attain this status can often be defined and is commonly regarded as the healing time for that fracture (Brasher 1965). The time taken for a fracture to unite may depend on factors such as age, the type of bone and the type of fracture. In early childhood, callus is often visible radiologically within two weeks and the bone may be united by six weeks. Union occurs a little less rapidly in older children. In adults, the union of a fractured long bone takes about 20 weeks on average particularly in the case of a large bone such as the tibia. Fractures through cancellous bone unite more rapidly than fractures through the hard cortical bone of the diaphysis. Thus, a fracture of the adult tibial shaft would be expected to be united in 20 weeks while fractures of the distal radius and ulna should be united in 8 weeks.

1.321 Delayed union

In clinical practice, a distinction is usually made between delayed union and non union. Delayed union occurs when it has taken longer than the expected time for a given fracture to unite (Brasher 1965). However, many such fractures will eventually unite without surgical
intervention. Clinically, an ununited fracture produces pain of varying intensity at the fracture site when stressed and on palpation, and there may be abnormal movement. There may be persistent oedema and increased local warmth. There is usually no visible continuity of bone to be observed on plain radiographs. Histologically, delayed union is characterised by fibrocartilaginous tissue at the fracture site (Sevitt 1981) but there is usually some evidence of ossification.

1.322 Non union

In established non union the reparative processes would appear to have come to a halt and it is envisaged that healing will not occur regardless of how much more time elapses. Clinically, non union often presents with little pain. There may be little movement at the fracture site although progressive angulation may occur particularly in weight-bearing bones. There is usually no true radiological continuity and a pseudarthrosis may be observed. Histologically, non union is characterised by dense fibrous tissues at the fracture site and the absence of a bony bridge. The fractured bone ends appear avascular (Verbeck & Bubbelman 1961, Hicks 1963) and may be covered by fibrocartilaginous tissue with the formation of a cyst in the fracture cleft, the so-called pseudarthrosis. Cell division and new bone formation (sclerosis) may be observed but the fracture cleft is not filled up or bridged (Urist & Johnson 1943, Wendeberg 1961). It is difficult in practice to identify a slowly healing fracture which will eventually develop non union and thereby require mandatory surgical
intervention.

1.32 Patho-radiological types of delayed and non union

Slow union of fractures usually presents as one of two broad pathological varieties (Fig. 7) based on the radiological appearances; viz., hypertrophic and atrophic (Judet et al. 1958, Brasher 1965, Weber & Chech 1976). In the hypertrophic variety (Fig. 7a), although callus or 'new bone formation' is present, often in abundance at either end of the fracture fragments, calcification of the fibrocartilaginous bridging tissue is not completed and the fracture gap is not bridged by bone. It has been suggested that this state of affairs is due to persistent motion at the fracture site (Hicks 1963) since most hypertrophic non unions eventually go on to bony union with continued external immobilisation or with rigid internal fixation (Crenshaw 1980). However, permanent fibrous union may ensue and the bridging tissue may develop degeneration and cavitation to form a pseudarthrosis.

In the atrophic variety of slow healing (Fig. 7b), no attempt at proper healing takes place, rather a scar tissue is formed similar to that which occurs in soft tissues. There is often no evidence of active osteogenesis and union is rarely achieved without bone grafting (Watson Jones 1943).

1.33 Causes of delayed union

Many tibial shaft fractures do not unite although they
have received treatment by currently accepted methods (Charnley 1970). There is usually no ready explanation for the failure of these fractures to heal promptly but several general and local factors have been proposed (Campbell 1939, Watson Jones 1943, Cruess 1984). Only rarely have systemic factors been shown to influence the speed of healing of fractures. For instance, lack of vitamin C may prevent the formation of collagen and bone and lack of vitamin D may cause failure of mineralization of the fracture callus. However, slow healing of tibial shaft fractures may, in fact, be commonest in many societies where nutrition is not a significant problem.

The balance of evidence currently available is that local factors are more important in the causation of slow healing of fractures. Many local factors have been proposed but the most important of these appear to be poor blood supply (Watson Jones 1943, Jackson & Macnab 1959, Compere 1949, Bach & Hansen 1983), severe concurrent soft tissue damage (Ellis 1958a, Bauer et al. 1962, Edwards 1965a & b, Hoaglund & States 1967), damaged periosteal envelope (Jackson & Macnab 1959, Pritchard 1961, Ham & Harris 1971), extensive bony injury (Henderson 1926, Urist et al. 1954, Nicoll 1964, Johner & Wruhs 1983), soft tissue interposition (Watson Jones 1943) and inadequate immobilization (Hicks 1963).

The importance of inadequate immobilization in the causation of delayed healing of fractures is often overdramatised and Hulth (1980, 1981) has recently challenged its validity. Clinical experience suggests that fractures at
sites such as the ribs and clavicle which are subjected to continuous motion have a lower prevalence of delayed healing compared to the tibia. In fact, many fractures are treated successfully using methods which provide poor immobilization. For instance, fractures of the femoral shaft are often successfully treated by Thomas's splint which allows significant movements at the fracture site. This suggests that inadequate immobilization per se may not be a serious factor in the retardation of fracture healing.

On the other hand, soft tissue interposition as a cause of delayed union may be important in fractures of bones heavily invested with muscles such as femoral shaft fractures. The tibia is subcutaneous along the whole of one of its borders and poorly invested with muscles in its lower half. Consequently, soft tissue interposition is seldom a problem in tibial shaft fractures.

1.331 Vascular damage as a cause of delayed union

The speed of union of fractures is probably closely related to the local blood supply since at sites where it is abundant such as the supracondylar region of the femur or distal end of the radius union rarely presents a problem. Furthermore, clinical experience indicates that fractures of cancellous bone heal faster than fractures of cortical bone which is thought to be less well supplied with blood. Many workers believe that provided fracture fragments have a good blood supply the fracture will heal. Consequently, in the early twentieth century several surgical (Andrews 1919) and
rehabilitation (Sinclair 1931) techniques were developed to increase blood flow to the fracture site.

Further evidence to support this view is provided by the high incidence of non union at sites where fracture is known to interrupt the blood supply to one fragment such as the head of the femur after fracture of the neck (Calandruccio & Anderson 1980). Surgical intervention is often mandatory to achieve union of fractures at such sites (Tovee 1953, Garden 1971) and healing occurs only if the living portions can incorporate the ischaemic fragments in the same manner as bone grafts (Glimcher & Kenzora 1979). It is obvious that healing will be slow under these circumstances compared to a situation where both fracture fragments are well supplied with blood (Hicks 1963). On the other hand, if all the fracture fragments are avascular, bony union cannot occur unless the fragments first become vascularised (Watson Jones 1943). Hence, of the several factors that may influence the rate of healing of fractures, the blood supply is usually regarded as the most critical (Watson Jones 1943, Compere 1949, Jackson & Macnab 1959).

Vascular damage is presumed to retard the speed of healing because it produces avascular necrosis of portions of the fracture fragments. Any surgeon who has performed bone grafting operations for delayed union must have been impressed by the avascular appearance in many cases. Histological examination often reveals tissue necrosis, detritus and excessive scar formation. Several authors regard this as evidence of tissue ischaemia (Urist et al. 1954, Ham
& Harris 1971) which presumably impedes osteoblast function. Dead bone contributes nothing to healing of fractures (Ham 1930, Ham & Harris 1971, Ham & Cormack 1979), therefore, segments of dead bone would have to be circumscribed or replaced by new living bone through the processes of bone resorption and bone deposition (Phemister 1930). Thus, the bigger the avascular segment the more the time it will take to effect its replacement with new living bone (Urist et al. 1954). Although some workers notably Phemister (1930) and Trueta (1963) consider the necrotic bone as the most important stimulus for callus formation, extensive bone necrosis has been shown to increase healing time (Phemister 1951, Hicks 1963). This is presumably due to necrosis spreading beyond a certain critical limit and consequently, changing a physiological stimulation into a physical impediment to fracture healing.

The blood supply to fracture fragments is important because osteogenesis depends upon the presence of oxygen. Ham (1930) drew attention to the differentiation occurring in the fracture callus. The cells closest to the shaft, which were supposed to be well supplied with blood, differentiated into bone while those furthest away and differentiating in a relatively avascular environment differentiated into cartilage cells. Bassett and Herman (1961), using tissue cultures from the tibial cortices of chick embryo, have shown that the differentiation of primitive connective tissue cells is significantly affected by the environmental oxygen concentration. They showed that cultures exposed to 35% oxygen formed osseous tissue but the same cells formed
fibrocartilaginous elements when exposed to 5% oxygen. Markby et al. (1967) investigating the effects of chronic hypoxia on the rate of fracture healing in rats found delayed union in animals not acclimated to low oxygen environment. Pertinen (1972) observed decreased mineralisation in similar circumstances and Vaes and Nichols (1962) have suggested that hypoxia lead to the accumulation of lactate which then suppressed osteogenesis.

However, although these vascularity hypotheses may explain what happens in a particular callus, they do not explain why in some calluses ossification is preceded by cartilage and in others it is not. Dunham (1978) in her work on the biochemistry of fracture healing pointed out that areas of intramembranous ossification were usually to be found adjacent to areas of endochondral ossification. The lack of transition between the two processes of ossification indirectly suggests that these are independent phenomena and, therefore, vascularity cannot be used to explain why differentiation proceeds along one direction or the other.

Furthermore, several pieces of evidence appear to cast doubt on the role of blood supply specifically in slow healing of shaft fractures. For example, results from a number of experiments would appear to suggest that delayed union may be present despite seemingly adequate vascularity. Ficat et al. (1984) using perfusion techniques reported marked vascularity at the site of delayed unions. Laurnen and Kelly (1969) reported four times normal blood flow as measured by strontium-85 clearance in a canine tibial shaft
osteotomy which was slow in uniting. Paradis and Kelly (1975) reported similar results using iodine-125 labelled 4-iodoantipyrine washout as a measure of blood flow. Brighton and Krebs (1972) using microelectrodes found higher oxygen tension at the site of delayed union in rabbits. Thus, delayed union can occur in the presence of excellent vascularity and blood flow, clearly establishing the importance of other factors. However, no studies appear to have been carried out to examine a cause and effect relationship between vascularity and slow healing. Therefore, this whole area of fracture healing deserves further study.

1.332 Soft tissue damage as a cause of delayed union

It is generally believed that severe damage to the soft tissues impairs fracture healing. In many case series, for example, open fractures are shown not to do as well as closed fractures. Carpenter (1966) showed that union becomes difficult if the soft tissues overlying the tibia are not preserved. Rehn (1923) demonstrated a direct relationship between soft tissue damage and the amount of callus formed at the fracture site. Several workers have described an association between the speed of healing and the severity of injury sustained by the soft tissues adjoining the fracture (Ellis 1958a, Bauer et al. 1962, Edwards 1965a & b). Further experimental support comes from Whiteside and Lexer (1978) who demonstrated significantly higher incidence of delayed union rates in rabbit osteotomies when tissue dissection was extraperiosteal and when muscles were transected.
However, the exact role played by the soft tissues in fracture healing is unclear. Nevertheless, it seems possible that the development of bone necrosis after fracture may be related to concurrent soft tissue damage because the normal blood supply to bone may be derived in part from the soft tissues. Zucman (1960) and more recently, Simpson (1985) have demonstrated an intimate relationship between the blood vessels of the muscles, periosteum and bone. Therefore, damage to the soft tissues after fracture may, at the very least, diminish the blood supply to the fracture fragments thereby creating a state of relative ischaemia. Under such circumstances, the proliferative activity of the osteogenic cells may be depressed (Vaes & Nichols 1962, Markby et al. 1967, Pertinen 1971) or their ability to differentiate directly to bone may be impaired (Ham 1930, Bassett & Herman 1961).

In addition, the new blood vessels formed in response to fracture repair develop from pre-existing vessels (Wray & Lynch 1959, Trueta 1963, Danckwardt-Lilliestrom 1969). It is accepted that the surrounding soft tissues contribute most of these new vessels (Gothman 1961, Pritchard 1961, Koekenberg 1963, Rhinelander 1974). Sinclair (1931) described how the blood supply proceeding centripetally from the periphery of the injured parts reached the soft tissues first, then the haematoma which it organised before attending to the bony cleavages. Thus, the more severe the damage sustained by the soft tissues the slower the speed of vascularisation of the fracture site, and the slower would be the speed of healing.
Furthermore, severe soft tissue damage is associated with widespread infiltration of the tissues with blood. This may result in extensive traumatic inflammation which could impair healing by the formation of scar tissue in the fracture area (Sinclair 1931). This may cause widespread soft tissue adhesions which may interfere with the vascular and osteogenic capacity of the periosteum. In addition, Pritchard (1961) believes that the contribution of the soft tissues to the healing process determined whether or not fracture repair was by fibrous or bony tissue. Damaged soft tissues may provide blood-borne polymorphonuclear leucocytes from which some workers believe that fibroblasts are derived (Becker & Murray 1967, 1970). McLean and Urist (1955) have proposed that these fibroblasts form the fibrocartilaginous tissue sometimes found in the fracture callus. On the other hand, Hulth (1980, 1981) has postulated that products from injured tissues stimulate the structural genes of undifferentiated cells to produce messenger RNA and proteins for either fibrous repair or bony repair depending on the severity of injury. The more severe the injury the more the likelihood of fibrous tissue repair, and although fibrous tissues can be converted to bone (Mulholland & Pritchard 1969, Sevitt 1981), this takes longer than direct bony union.

Damage to the soft tissues may play yet another role in fracture repair by contributing to the haemorrhage in the vicinity of the fracture. The more severe the soft tissue trauma the larger will be its contribution to the ultimate size of the fracture haematoma. This is very important since fracture haematoma may be a vital step in the healing process
(Gallie & Robertson 1920, Leriche & Policard 1928, Potts 1933). Becker and Murray (1967, 1970) have suggested that the fracture haematoma is the medium in which the early stages of repair take place. Some workers believe that the amount of new bone formed corresponds exactly to the amount of fracture haematoma. On the other hand, Ham (1930) and Sevitt (1981) propose that the fracture haematoma poses a physical impediment to healing. Whatever one's views, it is obvious, that even for purely physical reasons, a large haematoma will take longer to replace than a smaller one. The different hypothetical mechanisms of action attributed to soft tissue trauma may have elements which can be proved by experimental studies or they may remain only hypotheses but it is clear that injured soft tissues probably play a role in delayed union.

1.333 Periosteal damage as a cause of delayed union

In theory, there are many ways by which damage to the periosteum may delay the healing of a fracture. First, avulsion of the periosteum in the vicinity of fracture even when the soft tissues have not sustained major injuries may jeopardize the local blood supply (Sinclair 1931). The deprived bone may not die but the ends of the fractured bone may become ischaemic (Ham 1930, Sevitt 1981) and the local contribution to bone repair may be abolished or reduced to a minimum. Trueta and his colleagues (1955, 1964, 1965, 1968) demonstrated the importance of periosteal blood vessels in preventing delayed union and non union in undisplaced fractures of the canine radius. As long as the periosteal
vessels were intact, no delayed union resulted even when the intramedullary circulation was completely disrupted. Campbell (1939) claimed that the prominence of periosteal callus in fracture healing is due to its relatively more profuse blood supply. This apparently makes the periosteum the most easily accessible structure to the invading granulation tissue which enters the fracture site to organise the haematoma and form the substrate for new bone.

Second, the periosteum may play a role in isolating the fracture site from the fibrous tissue elements repairing the adjoining soft tissues. Macnab (1958) and Pritchard (1961) have suggested that it is the periosteal envelope that keeps the fracture site from being invaded by fibrous tissue ingrowths from the adjacent soft tissues. Thus, the extent of the periosteal injury at the moment of fracture may determine the amount of fibrous tissue within the fracture gap. Fibrous tissues only ossify slowly (Mulholland & Pritchard 1969, Sevitt 1981), therefore, their abundance within a fracture gap will retard the speed of healing of the fracture.

Third, most authors agree with Ham (1930) that the periosteum plays a crucial role in providing osteogenic cells for the repair of shaft fractures. Clinical experience suggests that fractures in which the periosteum has not been devitalised begin callus formation earlier than those in which there is widespread periosteal destruction (Aegerter & Kirkpatrick 1968).
However, if periosteal damage is important why is it that delayed union occurs infrequently in childhood although periosteal damage must also accompany fractures in this age group? In this connection, Charnley (1970) and Ham and Harris (1971) have made the interesting observation that the periosteum in children is more resilient and consequently, it is less likely to be shredded by violent injury. Therefore in childhood shaft fractures the periosteal sleeve may only be perforated by one of the fracture fragments so that a continuous periosteal tube is always to be found joining the main fracture fragments. Hence, fibrous union is rare in childhood.

Furthermore, the generally observed rapidity of healing in young individuals compared to adults is commonly held to be entirely a reflection of the differences in cellular response to fracture of the periosteum (Ham & Harris 1971). It is claimed that there is an intrinsically increased rate of cell proliferation in the young (Fischer 1946, Bourliere 1950). Du Nouy (1932, 1936) proposed that healing time was constant at a particular age and was related to the individual's physiological reparative activity. He expressed this mathematically and calculated that cell proliferation at 20 years of age was twice that at 40 years.

The periosteum is, of course, the site of an orderly and highly polarised intramembranous bone formation in the growing child (Ham & Cormack 1979). Consequently, it is thicker and adjudged more osteogenic during childhood. MacLean and Urist (1961) and Tonna and Cronkite (1961, 1962)
have confirmed that the periosteum of the young has a greater proliferative capacity than that of the old. The reason for this is not known but the harmful effects of cosmic irradiation and a fall in the synthesis of plasma growth factors or increase in synthesis of their inhibitors with age have all been proposed as reducing the osteogenic potency of adult periosteal cells (Kunze 1932, Failla 1960). However, it is not explained why the healing time for a particular fracture at age 20 is in practice not different from that at 60 years of age which suggests that factors other than age are involved. For example, age differences in the speed of healing are not as obvious in cancellous bone fractures as they are in shaft fractures.

In this connection it should be pointed out that the periosteum of the child is not only more cellular but is also more vascular than that of the adult and which of these factors is more important is not clear. It could equally be argued, and rationally, that the increased vascularity of the juvenile periosteum is responsible for the rapidity of healing of fractures in this age group. Perhaps both are equally important since biologically the greater the number of cells the greater the demand for blood supply so that cellularity may be inseparable from vascularity. The important consideration here is that not only is the pool of osteogenic cells potentially available for fracture healing greater in childhood than in adulthood, the potential sources of blood supply may be greater as well and this may account for the rapidity of healing of childhood shaft fractures.
Some authors have claimed that the extent of bony injury determines the speed of healing. In pathological terms, it has been suggested that the damage sustained by bone at the time of fracture is one of ischaemia (Ham & Cormack 1979, Sevitt 1981) due to tearing of the vessels within the bone and in its coverings. Histologically, this is manifested in the disappearance of osteocytes from lacunae within days of injury (Ham 1930, Sevitt 1981). The death of these cells is apparently important because it determines the requirement for repair.

It is commonly stated that the damaged areas of a fractured bone contribute nothing to the healing process (Ham 1930, Ham & Harris 1971, McKibbin 1978, Ham & Cormack 1979). Consequently, fracture healing probably involves the progressive circumvention of localised areas of injured bone with a bridge of new bone. If this were so, according to Urist and his colleagues (1954), then the more damage a bone sustains during fracture, the more extensive the repair has to be. Since fracture repair is a cellular function, repair will be time dependent so that a large bony defect would take longer to heal than a small bony defect (Urist et al. 1954). Thus, segmental and comminuted fractures would heal more slowly than simple transverse, oblique or spiral fractures. This indeed appears to be the clinical experience reported by several workers, the more comminuted a tibial shaft fracture is the higher the rate of complications and failure (Ellis 1958a, Bauer et al. 1962, Nicol 1964, Weissman et al. 1966,
When a comminuted fracture is sustained, for example, many pieces of the bone at the fracture site will probably be deprived of their blood supply because they have lost their soft tissue attachments. Inevitably, the osteocytes in these fracture fragments die so that in effect, the pieces of bone constitute an autologous implant or graft which requires to be freshly incorporated into the cortex during repair. This may be achieved by building a callus bridge to circumvent the ischaemic fragments as described above or by a process of re-vascularisation (Stringa 1957). However, cortical bone is only very slowly re-vascularised (Phemister 1930) and, therefore, some of the fracture fragments may remain avascular for considerable lengths of time. Thus, the larger the amount of cortical bone to revascularise the longer it would take for the fracture to heal. Even if a fracture fragment is still attached to some soft tissues, it is not clear whether it retains an adequate blood supply as suggested by Kuntscher (1968).
a. Haematoma concept (Gallie & Robertson 1920)

granulation tissue invasion of haematoma
conversion to fibrocartilage
conversion to bone

b. Periosteal concept (Ham 1930)

migrating callus collars
fused callus collars

migrating primary callus (periosteum)
inductive callus (fracture gap)
the fracture callus

c. Multiple origin concept (McKibbin 1978)

Figure 6 The principal concepts of fracture healing
Figure 7a
Plain radiographs of adult tibial shaft fracture showing hypertrophic non union
Figure 7b

Plain radiographs of adult tibial shaft fracture showing atrophic non union
1.4 PREVIOUS APPROACHES TO THE STUDY OF DELAYED UNION OF ADULT TIBIAL SHAFT FRACTURES.

As previously discussed, the factors which are responsible for the development of delayed union are not clearly understood nor can they be identified with any certainty in individual cases. Clinical parameters are not often reliable, for example, the chances of delayed union of a tibial shaft fracture are still considerable even with closed fractures and excellent end-to-end reduction (Charnley 1970). Some fractures designated as major injuries sometimes heal faster than apparently simple ones (Austin 1977) and the problem, therefore, is to identify those features of a fracture which would lead to delayed union. The accurate identification of such characteristics is vital in order to predict which fractures are at risk of developing delayed union and to allow suitable surgical action to be taken promptly in the hope of reducing the overall morbidity. A number of methods are being used at present to predict delayed union of fractures of the adult tibial shaft.

1.4.1 CLINICAL ESTIMATION OF SEVERITY OF FRACTURE

Two physical findings are generally used to identify slowly healing fractures at the outset based on a clinical perception of the severity of injury. For instance, open fractures are deemed to have sustained a more violent injury and more severe soft tissue damage than closed fractures.
Consequently, it is argued that compound fractures fare less well than closed fractures (Johner & Wruhs 1983) and the rate of delayed union may be as high as 90% (Carpenter et al. 1952, Sakellarides et al. 1964). Thus, in clinical practice fractures are usually classified as open or closed and efforts are made to quantify the fracture wound.

In the classification of compound fractures by Freedman and Ganes (1958) the first variety was a puncture wound created from within by the bone fragments penetrating the skin and this carried only a small risk of contamination with foreign material. The second type was a puncture wound compound from without in which contamination occurred but was minimal. In the most serious compound fractures, the laceration(s) was extensive and was potentially contaminated with foreign material. Gustillo and Anderson (1976) were more precise in their classification. Their Type I compound fracture was associated with a clean wound less than 1cm in length. The Type II wound was greater than 1cm in length but was not associated with extensive soft tissue damage or with tissue/skin flaps or avulsions. In Type III compound fractures, there was extensive soft tissue damage or traumatic amputation. Gunshot injuries, farm injuries and vascular injuries were regarded as special categories of Type III open fractures.

Clinical experience, however, suggests that there is no direct relationship between wound score per se and failure to unite. Dehne et al. (1961), Sarmiento (1967) and Hoaglund and States (1967) dismissed open wounds as being of any
importance to the speed of healing provided such wounds were properly managed. The exact role of open wounds in the pathogenesis of delayed union, therefore, remains unclear.

On the other hand, many closed fractures associated with severe soft tissue damage may have a poor prognosis (Gotzen & Haas 1984). Laboratory evidence of this has been provided by Edwards (1965b) who demonstrated prolonged healing times in fractures of the canine tibia following additional trauma to the soft tissues. Holden (1972) described the deleterious effects of muscle ischaemia on the speed of healing but the exact mechanism(s) are unknown. Nevertheless, muscle injury may lead to oedema and to swelling which, particularly when accompanied by bleeding, may produce a sharp rise in tissue pressures within the fascial compartments thereby further jeopardising blood flow to the soft tissues of the limb and also presumably to bone.

There are very few articles in the English literature that describe methods of assessing or quantifying the soft tissue damage which may accompany fractures. In the classification proposed by Oestern and Tscherne (1984), four grades of soft tissue damage associated with closed fractures were described. In grade 0, there was negligible soft tissue damage. Grade I closed fractures were associated with superficial abrasions or contusions caused from within. Grades 0 and I closed fractures were apparently caused by indirect violence and were usually not significantly displaced. Grade II closed fractures, which included transverse fractures with or without butterfly fragments,
oblique fractures and segmental fractures, were associated with deeper abrasions and local skin or muscle contusion and were usually caused by direct trauma. The so-called impending compartment syndrome was included in this category. Finally, in the grade III closed fractures, the skin was extensively contused and muscle damage was severe. This variety of closed fracture was caused by direct trauma. It was associated with wide displacements and comminution. Decompensated or frank compartment syndrome and vascular injuries were included in this category. But in practice this classification must be very subjective indeed.

Nevertheless, the classification by Oestern & Tscherne (1984) appears to suggest that fractures with different morphologies are associated with different patterns of soft tissue damage. In their classification, spiral fractures were associated with grades 0 and I soft tissue damage, transverse and oblique fractures with grade II and comminuted fractures with grade III soft tissue damage. This concept may be important in predicting fracture behaviour because fracture healing may depend on the type of soft tissue damage sustained as a result of fracture. However, this possibility of an association between fracture morphology and specific patterns of soft tissue damage does not appear to have been previously explored experimentally. This aspect of fracture healing could be studied by producing a spectrum of tibial shaft fractures in experimental animals using different deforming forces, and subsequently examining the type and extent of the associated soft tissue damage.
1.42 RADIOLOGICAL CLASSIFICATION OF FRACTURE

Many attempts have been made to classify fractures of the shaft of the tibia in order to predict prognosis. Ellis (1958a) utilised the degree of initial displacement, angulation, comminution and the presence of open wounds to identify three prognostic groups; minor, moderate and major severity. A minor severity fracture was undisplaced, not angulated and had only a minor degree of comminution and/or a minor open wound. A moderate severity fracture had more noticeable displacement or angulation with a small degree of comminution and/or a minor open wound. In a major severity fracture displacement was complete and the fracture was associated with major comminution and/or a major open wound. Fractures in Ellis' minor group united at 10 weeks on average with a delayed union rate of 2%. Moderate severity fractures united at 15 weeks on average with a delayed union rate of 11%. The major severity fractures took more than 23 weeks to unite on average and 60% of them united very slowly indeed.

Jackson and Macnab (1959) who quantified the degree of displacement rather more precisely came to a similar conclusion as Ellis (1958a). The two studies suggested that severe degrees of displacement, major comminution and extensive open wounds were evidence of major soft tissue damage. Most authors now believe the extent of this damage determines the speed of healing of fractures (Bauer et al. 1962, Edwards 1965a, Allum & Nowbray 1980, Johner & Wruhs 1983, Haines et al. 1984).
Nicoll (1964) used similar fracture characteristics as Ellis (1958a) which he graded and combined into a "predictive" system. He suggested that the extent to which "unfavourable factors" were combined in any fracture determined the "personality" and the inherent propensity for union. He proposed three grades for each "unfavourable factor" from none to slight to moderate to severe. The information was coded on Hollerith cards and analysed using data processing techniques. Combining the three factors, he came up with a classification which apparently made the outcome of any given fracture reasonably predictable. Edwards (1965a) further simplified the classification by the use of a Venn diagram. Nevertheless, these classifications are cumbersome and have never received wide application in clinical practice. Those who have used these methods have complained that the classifications do not accurately predict prognosis in individual cases because of large degrees of overlap and because many fractures fail to behave in the predicted way. Austin (1977) reviewed 8 of the major reports on fractures of the tibial shaft and demonstrated that the prognosis of the "severe" fracture was, in fact, not easily predicted. Some "severe" fractures united more quickly than some "minor" ones. He concluded that there were special factors apart from simple severity scores related to poor prognosis.

It was probably for these reasons that Weissman et al. (1966) based their classification entirely on one factor, the initial displacement of the fracture. They defined displacement as the separation between the longitudinal axis
of the fracture fragments in the horizontal plane. In a minimally displaced fracture the shift was less than a fifth of the width of the tibia (and the angulation less than $10^\circ$). Mild displacement was characterised by a shift of one-fifth to two-fifths (and an angulation of $10^\circ$ to $30^\circ$). Marked displacement had occurred where there was more than two-fifths of shift and in severe cases displacement was total with loss of contact between the fracture fragments. Minimally displaced fractures healed faster than markedly displaced fractures. Consequently, Weissman et al. (1966) proposed that initial displacement was the most accurate indication of the severity of the initial trauma. They suggested that angulation at the fracture site was important only as regards cosmesis. They did not accept that the degree of comminution or the presence of open wounds carried sufficient weight to be used in classification.

These results have been confirmed by Hoaglund and States (1967) who showed that fractures displaced the total width of the shaft healed more slowly than those with less displacement. However, this view is certainly not universal and clinical experience suggests that wide displacements are important mainly because they indicate instability. Such fractures not infrequently require several attempts at closed reduction when treated conservatively. The role that repeated manipulations may play in the failure of these fractures to unite on time cannot be ignored. It is also to be remembered that most fractures will have changed alignment during transportation to and within hospital. Consequently, any classification relying entirely on the degree of initial
displacement is inherently flawed unless radiological examination was carried out at the scene of accident before the patient was moved.

Other classifications of fractures of the shaft of the tibia are based on the location of fracture in the shaft. Many surgeons believe that fractures of the lower third are more indolent than fractures elsewhere in the shaft (Watson-Jones 1943). It is often suggested that this is due to a poorer blood supply to the distal region of the diaphysis (Jackson & Macnab 1959, Compere 1949, Wilson 1981). This is said to be because the area is devoid of adequate soft tissue cover from which the blood vessels for fracture healing may be derived. This view has now been refuted by most studies involving large numbers of patients (Johner & Wruhs 1983). Nicoll's work and subsequently many others since have shown that the site of fracture has no influence on the speed of healing. Furthermore, perfusion studies carried out by experienced workers such as Brookes (1971) have not revealed any regional variations in the blood supply of the tibia.

It is apparent from the foregoing account that different authors stress different clinical and radiological factors as being important determinants of the speed of healing (Austin 1977, Keller 1983). There are probably as many classifications as there are authors. In most orthopaedic units, however, treatment options are often based on entirely different considerations such as costs, availability of beds and social circumstances of patient.
Consequently, most reports emphasize the comparative results of different methods of treatment but very little information concerning the basic cause of delayed union.

Johner and Wruhs (1983) approached the problem differently and attempted to correlate fracture morphology with those factors generally considered important in determining fracture healing such as mechanism of injury (Bauer et al. 1962, Edwards 1965a, Allum & Howbray 1980), extent of bone involvement (Urist et al. 1954, Nicoll 1964), degree of soft tissue injury (Edwards 1965b, Hamza et al. 1971) and degree of displacement (Jackson & Macnab 1959, Weissman et al. 1966, Hoaglund & States 1967). The letters A, B and C were used to represent increasing degrees of comminution (simple, butterfly and comminuted respectively). The numbers 1, 2 and 3 represented the mechanism of injury - 1 included all spiral fractures while 2 and 3 represented fractures produced by direct impact. Subgroups 1, 2 and 3 were used to specify the anatomical location of fracture. The results were interpreted as showing clearcut differentiation between spiral and non spiral fractures as regards the speed of healing unlike the other factors under consideration such as location of fracture on the shaft. Only the presence or absence of open wounds had the same influence on the course of healing as the fracture morphology. However, this study was retrospective and involved different types of fractures and different methods of treatment.

It would be helpful if these results could be confirmed since at the present time there is no simple radiological
classification which can predict delayed union with any consistency. At present, it would appear that the radiological manifestations of injury as commonly perceived are not related to the speed of healing of tibial shaft fractures. This whole area needs to be studied afresh particularly the relationship between fracture morphology and the speed of healing. Ideally this should be done prospectively in a group of closed fractures treated by a single method. It is also necessary to investigate experimentally the relationship between radiological morphology and local soft tissue damage.

1.43 BONE-SEEKING RADIONUCLIDE UPTAKE AFTER FRACTURE

A number of attempts have been made to use bone-seeking radionuclides to follow the course of fracture healing in order to separate normally healing fractures from delayed union and non union. Wendeberg (1961), using radiostrontium, observed increased activity at the fracture site in some patients several years after injury. Significantly, however, he was unable to differentiate between pseudoarthrosis, complicated fractures and normally healing fractures because isotope uptake was not markedly different from one to another.

Green et al. (1971) used the rat tibia as their model for investigating the uptake of radionuclide after fracture. Four groups of fractures were studied; namely, fractures manually produced, fractures produced manually followed by repeated manipulations, fractures in association with
gastrocnemius transection and fractures with muscle interposition. Gastrocnemius section, but not repeated manipulations, delayed healing while muscle interposition produced non union. No differences were found in the uptake of $^{99m}$Tc labelled albumin and radiostrontium in normal union, delayed union and non union. Even more importantly, hypovascular areas of the diaphysis detected by perfusion were not detected by radionuclide imaging.

Illingworth and Schiess (1971) studied the uptake of radiostrontium 24 hours after fracture and found that this was of no prognostic value as regards the time taken for union to occur. Johanssen (1973) reported a similar disappointing outcome in a clinical study involving 39 patients. He followed the course of fracture healing by serial external counting after radiostrontium injection and found that increased activity localised preferentially at the fracture site. The ratio of uptake between fractured and unfractured bone reached a maximum between 8 and 32 days after fracture. Another peak was observed 8 to 45 days after the commencement of weight-bearing. He demonstrated a tendency for the uptake ratio to occur later with increasing complexity of fracture but no significant differences were observed between complicated and uncomplicated healing.

On the other hand, however, Segmuller et al. (1970), also using radiostrontium, have claimed that delayed union and non union have relatively higher isotope uptake ratios compared with normally uniting fractures. Muheim (1973) reported serial qualitative radioistrontium uptake in 10
patients comparing conservative and operative management. He observed a progressive and rapid increase in tracer uptake after conservative treatment over a 28 week period but tracer uptake progressively decreased after osteosynthesis. Two of the fractures developed non union and in both cases the tracer uptake was thought to be different from that of the fractures uniting normally. Puranen et al. (1975) obtained a total of 203 strontium profile readings in 68 fractured tibiae. They found a slower decline in uptake (i.e. more prolonged accumulation) in delayed union and non union (200 to 400%) compared to normal healing (150%). Lund et al. (1978) demonstrated significantly higher ratios of Tc99m-Sn-pyrophosphate activity in slowly healing fractures 6 weeks after injury.

Gummermann et al. (1978) produced a histographic representation of the count rates along the length of fractured rabbit tibiae. They collected sequential gamma camera images for five groups of fractures; namely, control - immobilisation, control - immobilisation plus periosteal stripping, simple fracture - osteotomy, delayed union - osteotomy plus periosteal stripping and non union - osteotomy plus periosteal stripping and methylmethacrylate interposition. At one week, two peaks were revealed with a valley centred on the fracture line but this picture was replaced at two weeks by a broad single or biphasic peak. The disappearance of the isotope activity peak apparently coincided with radiographic callus formation. Delayed union differed from normal union only temporally since the different peaks were recorded in both but at different time
intervals. By contrast, no reproducible sequence of radionuclide localisation was discernible in non union.

Jacobs et al. (1981) employed dynamic scanning to differentiate between "hypertrophic" non union (see Fig. 7a) which is thought to have increased vascularity (Judit et al. 1958, Brasher 1965, Weber & Chech 1976) and "atrophic" non union (see Fig. 7b) which is thought to be relatively avascular (Brasher 1965, Weber & Chech 1976). These authors believed that radionuclide uptake was in two phases; an early uptake phase due to tissue vascularity and a delayed uptake phase due to accretion or accumulation. Their patients were scanned 1 - 7 times during the course of healing. At each session, for 15 minutes following the injection of 15 mCi of Tc99m-labelled methylene diphosphonate they obtained 30 second sequential gamma camera images. The images were processed and enhanced by computer and the accumulation of activity in each region of interest was corrected for the differences in area. This was then plotted on a vertical axis against time on the horizontal axis. The activity at the fracture site was displayed on the upper curve and that at the control area on the lower curve. Radioactivity at the fracture site was often found to be over four times that of the control area. After an early rapid uptake, related more to vascularity, the curve became linear between 7.5 and 15 minutes after injection. This was presumed to be an indication of bone formation and, therefore, the percentage uptake of activity from 7.5 to 15 minutes was thought to measure precisely the rate of bone formation. The difference in percentage activity between fracture site and control
sites for normal, delayed and non union was determined and a
time related graph was contractd. The slope of the graph was
found to be different for different healing types. In normal
healing the slope was 3.09 or a net increase of 3% uptake per
month, 1.42% in delayed union and 0.5% per month for
fractures requiring bone grafts. The possibility of delayed
union was detectable as early as 6 weeks after fracture and
non union after 2 months. Gregg et al. (1984, 1986) were,
however, unable to confirm these findings in a similar study.

Auchincloss and Watt (1982) evaluated the role of
scintigraphy specifically in predicting fracture healing and
60 adults with tibial shaft fractures treated by non
operative methods were studied. Visual assessment and
computer analysis of early (perfusion) and 3-hour (accretion)
scintigrams were carried out at six weeks after injury. From
these, longitudinal profiles comparing two different areas of
the fractured bone and transverse profiles comparing the
fracture site with a corresponding area of the unfractured
opposite tibiae were constructed. Four scan types were
visually identified; viz, fusiform, focal, discrete and poor
uptake. Fractures displaying discrete and poor uptake scans
were adjudged to be slower healing. Four areas of interest
were selected to calculate a fracture ratio and an
osteoporotic ratio. Delayed healing was associated with a
fracture ratio less than 2.5:1 and/or osteoporotic ratio
greater than 2.5:1. These values were averages, however.
There was a spread of values between individual fractures so
that the fate of a particular fracture could not be
determined with certainty.
Gregg et al. (1983, 1984, 1986) also looked at the morphology of the static scintigrams to see whether it would provide some indication of the speed of healing. They observed generalised increased uptake throughout the fractured tibia within hours of injury with additional local increase at the fracture site in some cases. They also classified the static images but in those tibias not uniting normally all the observed patterns of uptake were represented. They observed 'cold spots', which may have indicated loss of blood supply, in relation to the fracture site in 10% of their cases. However, this did not bear any definite relationship to the normal progression to union.

Recently, an Edinburgh group (Smith et al. 1987) have refined the methods of Auchincloss and Watt (1982) and have been able to identify slowly uniting fractures. Each patient was scanned periodically during the course of healing and various indices were calculated from both early (dynamic) and late (static) scintigrams. Delayed union was associated with an uptake ratio of less than 1.3 two weeks after fracture when uptake over the fracture site was compared with uptake over an adjacent site on a static scintigram. Other indices were less discriminating. This study has been the most emphatic concerning the value of scintigraphy in the early diagnosis of delayed union and it is important that their results be confirmed before this expensive technique attains widespread use.

It is apparent from the foregoing account that
conflicting results have emanated from radionuclide uptake studies of fracture healing. No clearcut differentiation in isotope uptake has been consistently observed between normal, delayed and non-union using uptake ratios (Wedenberg 1961, Green et al. 1971, Johanssen 1973, Muheim 1973, Puranen et al. 1975) or graphic/pictorial representation of absolute counts (Gummermann et al. 1978, Auchinloss & Watt 1982). It is, therefore, necessary for these techniques to be further explored before they become commonplace in clinical practice.

1.44 OSTEOEDULLOGRAPHIC EVALUATION OF FRACTURE HEALING

A number of authors have reported on the use of intramedullary injection of a contrast medium or osteomedullography in the evaluation of healing of adult tibial shaft fractures. The use of this technique is based on two assumptions; first, that all fractures interrupt the longitudinal flow of blood within the medullary veins (Gupta et al. 1980) and second, that fracture union can be equated with the process of re-vascularization (Rhinelander 1974). Therefore, if an injected dye was found to cross the line of a fracture, re-vascularization on which osteogenesis depends was assumed to have occurred (Kaski 1971). Fractures which do not demonstrate venous flow through or around the fracture gap by 12 weeks are considered as not likely to heal on time and treatment as appropriate is advised (Puranen & Kaski 1974).

However, most reports appeared to suggest that osteomedullography was reliable only in indicating that a
fracture will eventually unite but that it could not be used to predict when the fracture was going to heal. Connolly et al. (1984) have recently questioned even this limited potential of osteomedullography. They carried out twice weekly examinations to assess healing of transverse osteotomies of thirteen canine radii. They delayed union in four dogs by wrapping the fracture ends with parafilm and were still able to demonstrate the flow of dye from the site of injection in the medullary cavity of the distal fracture fragment into the medullary cavity of the proximal fracture fragments after about five weeks of fracture thus suggesting extraosseous connections. In a parallel study of 85 patients with non union, these authors used osteomedullography to assess twenty fractures and found that the technique was not infrequently wrong in predicting which fractures would eventually heal without intervention.

The report by Connolly and his colleagues (1984) is important because osteomedullography is a minor surgical procedure which may require anaesthesia (Puranen & Kaski 1971, Gupta 1980). It is uncomfortable for the patient and is not without risk and therefore, its use can only be justified if it added precision to the diagnosis of delayed union. Consequently, more experimental work is needed to determine, for example, whether or not the medullary venous networks are completely interrupted by all fractures.
1.5 SUMMARY AND PURPOSE OF STUDY

Because adult tibial shaft fractures are common and because they often heal slowly, they are expensive to treat and associated with significant morbidity. Therefore, the precise identification of the fractures at risk is important. The vast literature on this subject atests to our lack of success in identifying factors that may influence the healing of tibial shaft fractures. This may be due in part to the fact that most series investigate a mixed population of fractures. Tibial shaft fractures in those under 16 years of age have a good prognosis and the healing of fractures with traumatic or operative wounds may be abnormal, therefore, these fractures could be said to belong to specific groups of their own and should be studied separately. Closed fractures, which are the basis with which all others should be compared, have seldom been studied separately and prospectively in man. To do so could be important because it eliminates two variables, that is, open wounds and surgical treatment. Such a study monitored by radiology, by radionuclide bone scintigraphy and by biochemistry is reported in this thesis.

The essence of fracture healing is the formation of new bone but the repair is initiated by migrating vessels and proliferating osteogenic cells. The membranes of bone (Ham 1930, Ham & Harris 1971, Ham & Cormack 1979) and the surrounding mesenchymal tissues (Urist et al. 1954, Young
1962, Trueta 1963, Owen 1970) are the most important sources of the healing tissue. Consequently, it may be argued that the severity of damage to these structures occurring at the time of fracture will determine how long it would take a fracture to heal, if at all. A review of the literature suggests that different fractures may have different speeds of healing. Therefore, it would be interesting to investigate whether different fractures produce different types of soft tissue damage, particularly to the periosteum and to the blood supply, to account for different healing times.

Ischaemia of bone, which may retard fracture healing, occurs as a result of fracture (Sevitt 1981). The repair of bone utilizes cellular processes which are heavily dependent on an abundant supply of blood. Consequently, a knowledge of the blood supply of bone is helpful in understanding the healing of fractures. The anatomy of the blood supply of the diaphyseal cortex has, however, not been satisfactorily resolved and, therefore, the important circulation in fracture healing is not known with any certainty. An attempt has been made to demonstrate the nature of the vascular arrangements in the diaphyseal cortex and to elucidate the relationship between the different possible sources of blood supply to the cortex and their respective contributions to fracture healing.

Attempts have been made to answer the following questions:

1. Can delayed union be predicted on the basis of clinical
2. Can delayed union be predicted on the basis of periodic measurements of serum levels of biochemical markers?

3. Can delayed union be predicted on the basis of periodic bone scintigraphy?

4. Can the radiological varieties of tibial shaft fractures observed in man be reproduced experimentally in rabbits, which have a similar bone circulation, so that these can be used to correlate directly fracture pattern with deforming force?

5. What is the nature of the local soft tissue damage caused by different types of fracture?

6. To what extent does the complete destruction of either the periosteum or the marrow affect the repair of a simple experimental fracture?

7. What are the true relative contributions of periosteal, epiphyseo-metaphyseal and marrow circulations to the nutrition of the diaphyseal fracture fragments?
2.0 CLINICAL STUDIES
2.1 AN INVESTIGATION OF THE NATURAL HISTORY OF THE HEALING OF CLOSED ADULT TIBIAL SHAFT FRACTURES TREATED BY CLOSED METHODS.

2.11 Introduction

There is no recent reliable information available in the English literature concerning the natural history of healing of closed adult tibial shaft fractures treated by closed methods. Most relevant studies are retrospective or concerned with mixed populations of fractures and treatments (Allum & Howbray 1980, Johner & Wruhs 1983). It would be particularly valuable to know what proportion of fractures not united at 12 to 20 weeks, when most surgeons might be tempted to operate (Souter 1969), eventually go on to develop established nonunion. Furthermore, the reasons for the apparent susceptibility of fractures of the tibial shaft to develop delayed union are unclear. Although many factors have been proposed as influencing the outcome of adult tibial shaft fractures, as discussed in the introduction to this thesis (section 1.3) there is no agreement as to which of these are the most important.

This may be due to differences in the make up of the population of fractures studied in different series. There are many variables involved in fracture healing, therefore, the selection of material for study is important. It is for this reason that this survey was undertaken and it sets out to examine the natural history of unilateral closed adult tibial shaft fractures treated by closed means. A number of the so-called prognostic factors were examined in order to
demonstrate their clinical relevance.

2.12 Methods

One hundred adult patients, who had sustained a closed unilateral fracture of the tibial shaft and were treated by closed methods, were studied prospectively at the Leicester Royal Infirmary between 1 August, 1985 and 31 July, 1987. Detailed clinical information was recorded for each patient on a special proforma with particular reference to age, sex, aetiology of trauma, fracture morphology, presence of intact fibula and time to union.

Choice of patients and fractures:

Patients older than 16 years of age with closed unilateral tibial shaft fractures being treated conservatively in plaster of Paris casts and/or braces of various kinds were chosen for the study. Compound fractures and fractures treated primarily by open reduction were excluded to reduce the number of variables.

Fracture definition:

Transverse fractures were defined as those fractures in which the fractured surfaces were approximately perpendicular to the longitudinal axis of the shaft. In oblique fractures the surfaces subtended an angle of approximately 45° to the longitudinal axis. Other longitudinally oriented fractures were classified as spiral fractures. Comminuted fractures, i.e.,
those with one or more intermediate fragments, were classified as transverse or spiral depending on the general orientation of the fractured surfaces (Alms 1961). All segmental fractures were classified as transverse fractures.

The initial radiograph was classified according to morphology and location of fracture in the shaft. For ease of reference, fracture morphology was identified as follows:

- Type I - transverse
- Type II - spiral
- Type III - oblique
- Type IV - comminuted supramalleolar.

The location of fracture on the shaft was noted after the limit of the tibial shaft had been set at the tuberosity proximally and at 2.5 cm above the distal articular surface of the tibia distally. The diaphysis was then divided longitudinally into equal thirds (Fig. 8). Location of fracture was determined by its lowest boundary in order to eliminate the so-called upper third-to-middle third and middle third-to-lower third fractures.

Assessment of severity of trauma:

A rough estimate of the magnitude of the deforming forces was made based on the definitions of other workers particularly Bauer et al. (1962) and Allum and Mowbray (1980). Road traffic accidents, falls from heights more than 6 feet, blows from very heavy objects and crushing were classified as high energy violence. Falls from ground level or from low heights, sports
injuries and bicycle accidents in which a motor vehicle was not involved constituted low energy violence.

**Definition of fracture union:**

A fracture was regarded as united when all external appliances including the so-called "protective braces" had been discarded and the patient was fully weight-bearing. Normal union was defined as union occurring within 20 weeks of fracture (Ellis 1958a, Nicoll 1964) and fractures not united at 20 weeks were regarded as showing delayed union.

**Treatment:**

Patients were under the care of 10 different consultants with fairly independent views about management of tibial shaft fractures. There was no agreed protocol of treatment but nearly all patients were admitted into hospital for at least three days after fracture for the baseline investigations. Fractures were manipulated under general anaesthesia when required and all patients were initially treated in a groin to toe plaster of Paris cast. This was converted to a below knee plaster of Paris cast or orthoplast tibial brace at variable times according to the surgeon's preference. More than 70% of the patients started weight-bearing in their plaster casts at six weeks. One consultant believed in earlier weight-bearing.

Although all patients were followed up fortnightly in a special Tibial Fracture Study Clinic where the various investigations were arranged, treatment decisions such as what...
displacement was or was not acceptable, plaster cast wedging, conversion of long leg plaster casts to braces etc., were made and carried out by the consultant team in charge of the patient.

Analysis of data and statistical methods:

All calculations were carried out using the vaxcluster computer system at the Computer Centre, University of Leicester. The system comprised of two vax 8600 and one vax 11/785 and the programmes were written in either basic or fortran. Standard statistical programmes contained in the Statistical Package for Social Sciences or SPSS (Norusis 1983) and Minitab Interactive Statistics Package (Ryan et al. 1985) were used to analyse the data. Graphs were plotted automatically with the Datagraf package (Leicester University Computer Centre User Manual 1987).

The differences between the two healing groups with respect to age, sex, causative violence, location of fracture on the shaft, fracture morphology and presence of intact fibula were tested statistically using the chi square ($x^2$) tests (Swinscow 1983). The data was analyzed by computer using the Minitab package (Ryan et al. 1985) and then subjected to a discriminant analysis using the SPSS package in order to establish whether single factors or linear combinations of factors thereof could be used to separate normal from delayed union (Norusis 1983). To this end, half of the data was used to generate the discriminant functions and the other half was used to test the results independently to see how precise the
procedure was in allocating cases to the healing groups.

2.13 Results

The age range was from 16 to 89 years (mean ± 1SD, 34.7 ± 18.9) and there were 72 males and 28 females, a ratio of more than 2 to 1. Seventy-one fractures were caused by low energy violence, commonly, sports injuries, and 29 by high energy violence, commonly road traffic accidents. Three fractures were located in the upper third of the tibial shaft, 39 in the middle third and 58 in the lower third. Thirty-nine of the 100 fractures were Type I, 28 were Type II, 25 were Type III and only 8 were Type IV. The fibula was not fractured in 29 cases.

Eighty-one fractures were united at 20 weeks and 19 developed delayed union. Of the 19 patients whose fractures were not united at 20 weeks, conservative treatment was continued in all but 4 cases who were operated upon because no further progress in healing was anticipated by their attendants.

The average age of patients with normal union was 34.8 ± 19.9 years (M ± 1SD) and the average age of patients with delayed union was 34.4 ± 14.6 years (M ± 1SD). Four of 28 females (14.3%) and 15 of 72 males (20.8%) developed delayed union. There was no significant difference in these parameters between normal and delayed union.

The influence of the causative violence on the speed of healing is shown in Table 1. Ten of 29 (34.5%) fractures
associated with high energy violence developed delayed union compared to 9 of 62 (12.7%) fractures associated with low energy violence. The chi square test suggested that the difference between the two healing groups with respect to magnitude of violence was probably significant (0.02>p>0.01).

The influence of location of fracture in the shaft on the speed of healing is shown in Table 2. Seven fractures in the middle third (17.9%) and 11 fractures in the lower third (19.0%) developed delayed union compared to 1 of 3 upper third fractures (33.3%). There was no statistically significant difference between the two healing groups with regards to their relationship with the fracture location.

The influence of fracture morphology on the speed of healing is shown in Table 3. Seven of 39 (17.9%) Type I fractures developed delayed union compared to 6 of 28 (21.4%) Type II and 6 of 25 (24.0%) Type III fractures. No Type IV fracture developed delayed union. There was no statistically significant difference between the two healing groups with regards to their relationship with the fracture morphology.

The influence of the intact fibula on the speed of healing is shown in Table 4. Only 1 patient with an intact fibula (3.4%) developed delayed union. When subjected to the $x^2$ test, the difference between normal and delayed union with respect to the intact fibula was probably significant (0.02>p>0.01)

Discriminant analysis (Norusis 1983) of the data yielded
no individual factor(s) or linear combinations of factors which could be used to separate normal from delayed union.

2.14 Discussion and conclusions

This prospective survey has shown that the prevalence of delayed union, as defined, in a series of adult tibial shaft fractures treated by closed means, was 19% at 20 weeks. This compares favourably with the prevalence rates reported in three recent prospective series of mixed tibial shaft fractures (Auchincloss & Watt 1982, Gregg et al 1983, Haines et al. 1984).

The basic characteristics of the fracture population were not unusual compared to different large unselected series reported in the English literature in the last four decades (Ellis 1958a, Bauer et al. 1962, Nicoll 1964, Weissman et al. 1966, Allum & Mowbray 1980, Johner & Wruhs 1983). More fractures were sustained in the lower third of the tibia (58%) than anywhere else. The severity of trauma was related to the radiological fracture morphology in that high energy violence commonly caused transverse and oblique fractures whereas low energy violence commonly caused spiral and comminuted supramalleolar fractures ($x^2=13.04, DF=3, 0.01>p>0.001$). The severity of trauma also appeared to determine whether the fibula was fractured as well since low energy violence caused fewer concurrent fractured fibula compared to high energy violence ($x^2=2.74, DF=1, 0.10>p>0.05$).

An analysis of associated factors revealed no individual
factor or linear combinations of factors which could be used to identify an individual fracture as likely or not likely to heal on time. Ellis (1958a), Bauer et al. (1962) and, more recently, Allum and Mowbray (1980) have proposed that the speed of healing decreases with increasing severity of causative violence. In this study, a noticeable difference was observed in fracture healing between high and low energy violence. High energy violence was associated with a 34.5% delayed union rate compared to 12.7% with low energy violence (0.02 > p > 0.01). This is to be expected since the soft tissues surrounding the fractured bone are likely to sustain a more severe damage with high energy violence. However, when subjected to discriminant analysis, the factor of causative force could not separate normal from delayed union. This may be due to the fact that classification of causative force into high or low energy is an oversimplification of a complex event.

Some authors believe that fracture location in the shaft plays a significant role with regards to union (Ellis 1958a, Allum & Mowbray 1980). In this series, excluding fractures of the proximal third which are too few for any meaningful conclusions, there appears to be little difference in the speed of healing between fractures located in the lower third and those located in the middle third. Similar findings have been reported by Nicoll (1964) and by Sarmiento (1970) in case series which include open and closed fractures.

In addition, the present series does not confirm the often expressed opinion that transverse fractures take the longest to unite (Charnley 1970, Johner & Wruhs 1983). On the
contrary, the delayed union rate was similar in Types I, II and
III fractures which were through the hard compact bone of the
diaphysis. Thus, differences in prognosis which have been
observed in other studies between transverse and spiral
fractures may be due to factors which are not present in closed
fractures.

There is no evidence from this survey that the presence
of an intact fibula delays healing as claimed by Jackson and
Macnab (1959) and by Hoaglund & States (1967). Burwell (1971)
considers displaced isolated tibial shaft fractures an absolute
indication for open reduction and internal fixation. In this
series, only 3.4% of fractures with intact fibula developed
delayed union compared to 25.4% in the group with fibula
fractures (0.02>p>0.01). Our findings are similar to those
reported by Allum and Howbray (1980) and by Hooper et al.
(1981). It is possible that the favourable prognosis is due to
the fact that most of the isolated tibial shaft fractures were
caused by low energy violence. However, discriminant analysis
did not show this combination to be a useful prognostic
indicator.

The emphasis in this survey has been placed on the
severity of causative trauma, location of fracture on the
shaft, radiological fracture morphology and presence of intact
fibula. The degree of initial displacement was not analyzed
because fracture displacement at presentation is not synonymous
with displacement at time of injury which is more relevant. No
attempt has been made to correlate mechanism of accident with
fracture morphology as this is impracticable in the clinical
setting (Alms 1961). None of the parameters examined in the series proved to be precise in its association with normal or with delayed union. Two conclusions may be drawn from this observation. First, that the clinical factors often cited by many authors as contributing to delayed union are relatively unimportant or are at best marginal. Second, that no fundamental biological differences exist between most normally uniting and slowly uniting fractures.

This study has also shown the natural history of slowly healing closed adult tibial shaft fractures treated by closed methods. Of the 19 fractures not united at 20 weeks, conservative treatment was continued successfully in 15 patients and their fractures healed before 30 weeks. Only 4 fractures were operated upon because their attendants believed that no further progress in healing was likely. Thus, the chances that a closed adult tibial shaft fracture treated by closed means not united at 20 weeks will be united at the end of 30 weeks is better than 5:1. This is an important finding because it answers the question that is frequently asked by patients and surgeons alike about the chances of a fracture eventually uniting if it had not already done so at 20 weeks. Many patients who have invested time in conservative treatment often do not want operation.

It is not surprising that many so-called slowly healing fractures healed by 30 weeks on continued conservative treatment because in many cases the decision to continue immobilisation depended on other considerations apart from clinical union. These results contradict those of Lucas and
Todd (1973) who reported 12% delayed union at 32 weeks in a series of closed tibial shaft fractures. The results suggest that, with regards to healing, open reduction and internal fixation is rarely justified in closed adult tibial shaft fractures. More importantly, the results also provide a baseline data against which the results of other methods of treatment could be compared.
Table 1: Influence of causative violence on speed of healing.

<table>
<thead>
<tr>
<th></th>
<th>Normal union</th>
<th>Delayed union</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>High energy violence (n=29)</td>
<td>19</td>
<td>65.5</td>
</tr>
<tr>
<td>Low energy violence (n=71)</td>
<td>62</td>
<td>87.3</td>
</tr>
<tr>
<td>Total (n=100)</td>
<td>81</td>
<td>81.0</td>
</tr>
</tbody>
</table>

\[ x^2 = 6.36, \text{DF}=1, \ 0.02>p>0.01 \]

Table 2: Influence of fracture location on speed of healing.

<table>
<thead>
<tr>
<th></th>
<th>Normal union</th>
<th>Delayed union</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Upper third (n=3)</td>
<td>2</td>
<td>66.7</td>
</tr>
<tr>
<td>Middle third (n=39)</td>
<td>32</td>
<td>82.1</td>
</tr>
<tr>
<td>Lower third (n=58)</td>
<td>47</td>
<td>81.0</td>
</tr>
<tr>
<td>Total (n=100)</td>
<td>81</td>
<td>81.0</td>
</tr>
</tbody>
</table>

\[ x^2 = 0.43, \text{DF}=2, \ 0.9>p>0.8 \]
Table 3: Influence of fracture morphology on speed of healing.

<table>
<thead>
<tr>
<th>Type</th>
<th>Normal union No (%)</th>
<th>Delayed union No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I (n=39)</td>
<td>32 (82.1)</td>
<td>7 (17.9)</td>
</tr>
<tr>
<td>Type II (n=28)</td>
<td>22 (78.6)</td>
<td>6 (21.4)</td>
</tr>
<tr>
<td>Type III (n=25)</td>
<td>19 (76.0)</td>
<td>6 (24.0)</td>
</tr>
<tr>
<td>Type IV (n=8)</td>
<td>8 (100.0)</td>
<td>0 (00.0)</td>
</tr>
<tr>
<td>Total (n=100)</td>
<td>81 (81.0)</td>
<td>19 (19.0)</td>
</tr>
</tbody>
</table>

$x^2=2.42$, DF=3, 0.5<p<0.4

Table 4: Influence of intact fibula on speed of healing.

<table>
<thead>
<tr>
<th></th>
<th>Normal union No (%)</th>
<th>Delayed union No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact fibula (n=29)</td>
<td>28 (96.6)</td>
<td>1 (3.4)</td>
</tr>
<tr>
<td>Fractured fibula (n=71)</td>
<td>53 (74.6)</td>
<td>18 (25.4)</td>
</tr>
<tr>
<td>Total (n=100)</td>
<td>81 (81.0)</td>
<td>19 (19.0)</td>
</tr>
</tbody>
</table>

$x^2=6.42$, DF=1, 0.02<p<0.01
2.2 AN INVESTIGATION OF CHANGES IN SERUM BIOCHEMISTRY FOLLOWING CLOSED ADULT TIBIAL SHAFT FRACTURES TREATED BY CLOSED METHODS.

2.21 Introduction

Calcium and inorganic phosphate are important constituents of bone, forming the greater part of its mineral content, and are also present in small amounts in the serum. Alkaline phosphatase is the generic name for a group of enzymes widely distributed in the body especially in the liver, bone, placenta and intestine. (Lattner 1975, Smith 1979, Kaplan & Pesce 1984, Whitby et al. 1985). In bone, alkaline phosphatase is produced by osteoblasts during bone formation (Robinson 1923) and maintenance of mature bone. It is also present in the serum in small amounts. Changes in the serum levels of these three biochemical parameters often occur in association with various disorders of bone (Lattner 1975, Smith 1979, Whitby et al. 1985). Consequently, it is reasonable to suppose that their serum levels could change after fracture and that these changes may be different in patients with normally uniting fractures compared with patients with delayed union. It is over thirty years since serial biochemical changes were examined during fracture healing (Urist et al. 1954). Since then, there has been considerable improvement in the sensitivity and accuracy of measurement of these biochemical parameters. Therefore, it has become necessary to repeat these studies to determine whether changes occur in the base biochemical parameters after fracture and whether they are of prognostic importance.
Tibial fractures are usually produced by significant trauma which concurrently damages the surrounding muscles. As discussed in the introduction to this thesis (section 1.4), there are at present no satisfactory methods of assessing this. Creatine phospho-kinase (CPK) is a recognised and easily measured marker of skeletal muscle damage (Lattner 1975, Smith 1979, Whitby et al. 1984) and, therefore, its measurement in the serum of patients with fractures could provide a sensitive and specific test. Some workers have indeed proposed that the level of its rise may reflect the initial severity of muscle damage and, therefore, of the causative trauma (Larsson & van der Linden 1981). However, the report by these authors was in the nature of a preliminary communication only and their results are yet to be confirmed by others.

It has also been suggested that traumatic stress lowers the serum inorganic phosphate levels by a pre-renal mechanism (Lennquist et al. 1979, Loven et al. 1982). Nordstrom et al. (1977) studied serum inorganic phosphate fluxes in 17 severely burned patients. They observed an immediate rapid decrease which was followed by a more progressive fall until the fifth day. From there the value began to rise returning to the pre-trauma levels at two weeks. These authors appear to suggest that there is a relationship between severity of injury and the initial fall in inorganic phosphate levels. A similar study has not been carried out in fracture patients and, therefore, it is not known whether the severity of fracture could be determined by measurement of the serum inorganic phosphate levels.

Many experimental and clinical studies have suggested
that the circulating levels of growth hormone may determine whether a fracture healed on time. Hsu and Robinson (1969) demonstrated that apituitary dwarf mice produced only unmineralised soft callus with delayed union following manually produced tibial shaft fractures. Koskinen (1967) treated 64 patients with growth hormone for anticipated delayed union and reported beneficial effects. Misol et al. (1971) reported delayed union of a femoral shaft fracture in a patient with low circulating levels of growth hormone. Although there is no evidence to suggest that patients with delayed union are pituitary deficient, some authors believe that trauma may lead to a state of temporary insufficiency (Bostrom et al. 1971).

Recently, Coates et al. (1981) reported decreased serum somatomedin (SM) activity in burned patients occurring within hours of injury and lasting more than a week. The duration of depressed activity correlated with the area of burns and with the degree of hypovolaemia. Similar findings were observed in the plasma of so-called mechanically injured patients (Coates et al. 1982) and the SM levels apparently correlated with the injury severity score and with the probability of death. Since increased tissue trauma is associated with delay in fracture healing, it is possible that this is mediated through depressed SM production. If SM plays a central role in normal bone growth as suggested by several workers, notably Koskinen et al. (1978), then it is possible that delayed healing of fractures could be associated with decreased levels (Lindholm et al. 1977). This has been denied by Ashton and her colleagues (1986). This controversy needs to be resolved because if deficiency of growth hormone function is the basic defect in
delayed union, then it may be easily rectified by appropriate medication.

Hauschka et al. (1975) and Price et al. (1976) discovered a vitamin K-dependent protein called osteocalcin, which is thought to be specific for bone. Osteocalcin is a 49 amino acid protein containing γ-carboxyglutamic acid. It has a molecular weight of 5,800 and constitutes approximately 25% of the non-collagenous proteins. It is thought to be synthesized by the osteoblasts under the influence of 1,25 dihydroxy vitamin D. Once secreted it apparently binds strongly to hydroxyapatite (Price et al. 1980). It circulates in blood and its serum level is thought to reflect accurately the bone formation rate (Brown et al. 1984). Direct experimental evidence suggest that the osteocalcin measured in the serum is newly synthesized and is, therefore, a reflection of osteoblastic activity (Price et al. 1981). Thus, if delayed union is due to abnormalities of osteoblastic function, this could be reflected in the the serum levels of osteocalcin.

In this study, sequential measurements of serum CPK, calcium, inorganic phosphate, alkaline phosphatase, creatinine, osteocalcin and somatomedins have been carried out throughout a 20 week period following fracture. An attempt was made to use the results to differentiate high from low energy violence fractures and normal from delayed union. High energy violence was assumed to be associated with more severe soft tissue damage compared with low energy violence. Creatinine levels were measured to indicate renal function because its impairment can considerably influence the serum inorganic
phosphate levels (Lattner 1975, Kaplan & Pesce 1984).

2.22 Methods

Aliquots of serum were obtained from 54 of the 100 adults with closed unilateral fractures of the tibia being treated by closed methods at the Leicester Royal Infirmary between 1 August, 1985 and 31 July, 1987.

Drug treatment:

The patients were treated with a variety of drugs throughout the period of study including intramuscular omnopon or pethidine and entonox or other anaesthetics. Some patients were transfused with various plasma expanders and others had intravenous Hartman's solution, saline and/or dextrose-saline as required. No attempt was made to group patients according to medication or according to the routes by which these were given since there is no evidence to suggest that these manoeuvres could have more than a marginal effect on results.

Routine biochemical estimations:

Serum was obtained daily for the first three days after fracture and, thereafter, fortnightly for twenty weeks. 10ml unclotted blood was obtained by venepuncture and submitted for total CPK determination by the N-acetyl cysteine - ethylene diamine tetracetate (NAC-EDTA) activated method (Kaplan & Pesce 1984). Phospho-creatine is used to generate nicotinamide adenine dinucleotide (NADPH) (Merckostest R Kit), the rate of
increase of which is determined photometrically at 340nm using the Centrifuchem 400 Centrifugal Analyser. This is directly proportional to the CPK activity in the sample.

The sample was also used to measure the calcium, inorganic phosphate, total alkaline phosphatase and creatinine levels using the Sequential Multiple Analyser with Computer (SNAC II System). For inorganic phosphate, the serum was dialysed with sulphuric acid, and ammonium molybdate was added to form a phospho-molybdate complex. This gives a blue colour in the presence of a reducing agent the intensity of which is related to the quantity of inorganic phosphate in the sample. Calcium was measured after mixing the diluted sample with 0.25N hydrochloric acid (HCl) to release the protein-bound calcium. It was then dialysed into a receipient stream of 0.25N HCl and reacted with cresolphthalein containing 8-hydroxyquinoline, which binds magnesium, and with diethylamine. The pink colour formed is proportional to the amount of calcium in the sample. All calcium levels were adjusted for variation in serum albumin levels by 0.025 mmol/l for each gram above 40gm/l. For alkaline phosphatase measurements, para nitrophenol phosphate, which is colourless, was added to serum to generate paranitro phenol, a yellow solution. This was quantitated at 410nm and the intensity of colour is proportional to the alkaline phosphate activity in the sample. Creatinine measurement was based on the reaction of saturated picric acid with creatinine in an alkaline medium (Jaffe reaction). The product was then measured at 505nm.

Osteocalcin estimations:
The serum level of osteocalcin was also measured using venous blood obtained from 7 patients with normal union and 7 with delayed union at four weekly intervals, from 0 to 16 weeks; a total of 5 samples per patient. The number of specimens examined was limited by financial considerations.

The serum was extracted and deep frozen until assay. Osteocalcin was measured by a radioimmunoassay procedure using the OSTK-PR kit (Compagnie ORIS Industrie). This assay is based on competition between $^{125}\text{I}$ labelled osteocalcin and osteocalcin contained in standards and in samples to be assayed, for a fixed and limited number of antibody binding sites (see appendix 1). The sensitivity of the assay determined by the kit manufacturers is 0.35 ng/ml and the mean ($\pm$ 1SD) value for normal subjects is 5.3 ($\pm$ 1.7) ng/ml. All values for standards and samples were determined in duplicate.

Somatomedin estimations:

Aliquots of serum obtained from 14 patients with normal union and 13 with delayed union shortly after fracture and, thereafter, every two weeks for 6 weeks, a total of 4 samples per patient, were also used to determine the somatomedin levels.

The serum was extracted and deep frozen until assay. Somatomedin activity was measured by a radio-immunoassay method using the IN-SONC kit (Compagnie ORIS Industrie). The assay is based on competition between $^{125}\text{I}$ labelled SM and somatomedin-C
(SMC) contained in standards and in samples to be assayed, for a fixed and limited number of antibody binding sites (see appendix 2). The sensitivity of the assay determined by the manufacturers is 2 nmol/l and the lower limit of normal for adult subjects is 9.09 nmol/l. All values for standards and samples were determined in duplicate.

Analysis of data and statistical methods:

Fractures were grouped according to severity of trauma, into low and high energy violence groups (Bauer et al. 1962, Allum & Nowbray 1980), and according to speed of healing, into normal and delayed union (Ellis 1958a, Nicoll 1964).

Results were statistically compared using the chi square ($\chi^2$) test and non parametric methods (Sinscow 1983). Graphs were drawn with standard deviation (SD) bars to emphasize the spread of values using the datagraf package (Leicester University Computer Centre User Manual 1987).

For ease of interpretation of results, biochemical values obtained at Day 0, 1, 2 and 14 were separately analyzed for severity of trauma and speed of healing and those obtained at Week 0 to Week 20 were analyzed for speed of healing only.

2.23 Results

In practice serum measurements could not be obtained at each planned interval for every patient for reasons beyond the control of the author. A number of patients objected to giving
blood after their fractures had healed. Others absconded because these investigations were not being used directly in making treatment decisions. Some specimens were lost in transit to the laboratory and other results were lost because of failure of equipment in the laboratory.

The age range of patients was from 16 to 82 years (mean ± 1SD = 30.7 ± 14.4). There were 13 females and 41 males. The fracture was united in 41 patients at 20 weeks and 13 patients developed delayed union.

**Injury phase biochemistry:**

The mean values of serum CPK in the first 14 days following fracture caused by low and high energy violence are shown in Table 5 and graphically in Fig. 9. A rise in the serum CPK above the normal level of less than 200 iu/l occurred within hours of fracture in most cases. The rise was significantly higher for high energy violence compared with low energy violence at one (p=0.012) and at two days (p=0.001) following fracture.

The mean values of serum inorganic phosphate in the first 14 days following fracture caused by low and high energy violence are shown in Table 6 and graphically in Fig. 10. The normal range is 0.8 - 1.4 mmol/l and nearly all the measurements made were within this limit. A rise in the serum levels of inorganic phosphate from baseline values (obtained within 24 hours of fracture) occurred in all cases. The mean of the baseline values was found to be higher after high energy
compared with low energy violence but this was not statistically significant.

The mean values of serum calcium in the first 14 days after fracture are shown in Table 7 and graphically in Fig. 11. The normal range is 2.0 - 2.60 mmol/l and all the measurements made were within this range. A rise in the serum levels from baseline values (obtained within 24 hours of fracture) occurred in all cases but fractures due to high energy violence appeared in general to have lower values. The difference between the two trauma groups was statistically significant within 24 hours after fracture (p=0.021) and at one (p=0.042) and at fourteen (p=0.031) days respectively following injury.

The mean values of total serum alkaline phosphatase in the first 14 days after fracture are shown in Table 8 and graphically in Fig. 12. The normal range is 40 - 130 iu/l. The range of values measured was very large and there was no statistically significant difference between low energy and high energy violence.

The mean values of serum creatinine in the first 14 days following fracture by low and high energy violence are shown in Table 9 and graphically in Fig. 13. The normal range is 60 - 120 iu/l. The range of values measured was very large and there was no significant difference between the two trauma groups.

The mean values of serum CPK in the first 14 days after fracture in normal and delayed union are shown in Table 10 and graphically in Fig. 14. There was a rise in the serum CPK above
the normal values within hours of fracture. The rise was higher for delayed union particularly on Day 2 after fracture but this was not statistically significant.

The mean values of serum inorganic phosphate in the first 14 days after fracture in normal and delayed union are shown in Table 11 and graphically in Fig. 15. There was a similar rise from the baseline values in both healing groups. The values were lower in delayed union at Day 0, 2 and 14 respectively after fracture but this was not statistically significant.

The mean values of serum calcium in the first 14 days after fracture are shown in Table 12 and graphically in Fig. 16. Although all measurements were within the normal limit, normally uniting fractures appeared to have the higher values and this was statistically significant at Day 0 (p=0.012) and at Day 2 (p=0.005) after fracture.

The mean values of total serum alkaline phosphatase in the first 14 days after fracture in normal and delayed union are shown in Table 13 and graphically in Fig. 17. The range of values was very large and there was no statistically significant difference between the two healing groups.

The mean values of serum creatinine in the first 14 days after fracture are shown in Table 14 and graphically in Fig. 18. The range of values was also very large and there was no significant difference between normal and delayed union.

Healing phase biochemistry:
The mean fortnightly values of serum CPK for normal and delayed union over a 20 week period are shown in Table 15 and graphically in Fig. 19. The trend was similar in the two groups. There was a fall to normal levels within 2 weeks from the high values obtained immediately following fracture.

The mean fortnightly values of serum inorganic phosphate for normal and delayed union are shown in Table 16 and graphically in Fig. 20. There was a rise from the baseline levels obtained immediately following fracture which peaked at 2 weeks and, thereafter, the values declined progressively over the 20 week period. Although the trend was similar for both groups, after 6 weeks normally uniting fractures appeared to have slightly higher values. The difference between the two groups was statistically significant at 16 weeks (p=0.020).

The mean fortnightly values of serum calcium in normal and delayed union are shown in Table 17 and graphically in Fig. 21. There was a rise from the baseline levels obtained immediately following fracture and, thereafter, the values remained at the upper limit of normal throughout the period of observation. The trend was similar for both healing groups but normally uniting fractures appeared generally to have higher values. The difference between the two healing groups was probably statistically significant at 14 weeks (p=0.053).

The mean fortnightly values of total serum alkaline phosphatase in normal and delayed union are shown in Table 18 and in Fig. 22. There was no statistically significant
difference between the two healing groups.

The mean fortnightly values of serum creatinine over the 20 week period in normal and delayed union are shown in Table 19 and in Fig. 23. There was no statistically significant difference between the two healing groups.

The mean values of serum osteocalcin at each interval of measurement are shown in Table 20. All values were within the normal reference range reported by several workers (Price et al. 1980, Brown et al. 1984, Epstein et al. 1984). Osteocalcin levels were lower in delayed union compared to normal union at each interval of measurement and this difference was probably significant at 8 weeks (p=0.050) and definitely significant at 16 weeks (p=0.0010). The results are graphically displayed in Figure 24 in which the slow healing fractures are represented by the lower curve. The curve for normal union shows an initial steady fall until the 12th week. By contrast, the curve for delayed union reveals no appreciable changes from measurement to measurement.

Plasma somatomedin activity was normal in all the samples obtained shortly after fracture and was practically unchanged from one interval of measurement to another for each patient. Only one measurement (8.0 nmol/l), at 6 weeks, for a patient who sustained a high energy fracture and subsequently developed delayed union, was below the lower limit of normal for adults. The highest value, 48 nmol/l, was also recorded for a patient with delayed union from serum obtained shortly after fracture. This patient had sustained low energy trauma. The mean
fortnightly values of serum somatomedin activity for low and high energy violence in the first 6 weeks after fracture are shown in Table 21. Fractures caused by high energy violence appeared to have lower SM activity shortly after fracture and at two weeks than fractures caused by low energy violence. However, this difference was not statistically significant. At 4 and at 6 weeks, the mean SM values were similar. The results are graphically shown in Fig. 25. The mean fortnightly values of SM activity for normal and delayed union in the first 6 weeks following fracture are shown in Table 22. SM activity in the serum of patients with delayed union appeared to have declined at the 6th week interval. However, there was no statistically significant difference between the two healing groups at any interval of measurement. The results are graphically analysed in Fig. 26.

2.24 Discussion and conclusions

This study demonstrates that CPK activity in the serum of patients with tibial shaft fractures increases significantly immediately after fracture (p<0.001). Fractures due to high energy violence have significantly higher levels than fractures due to low energy violence at Day 1 (p=0.012) and at Day 2 (p=0.001) after fracture. Therefore, CPK levels may truly reflect the degree of muscular injury, and hence, of soft tissue damage, following fracture. In addition, the mean value of serum CPK in delayed union is greater than that for normal union. Thus, high energy violence and delayed union are associated with higher CPK levels compared with low energy violence and normal union respectively. This is an important
finding because it appears to confirm the often expressed clinical impression that delayed healing may be closely related to degree of soft tissue damage (Ellis 1958a, Bauer et al. 1962, Edwards 1965a, Hoaglund & States 1967, Allum & Mowbray 1980). It raises the possibility that serum CPK levels immediately after fracture could be used as an aid to predict prognosis.

Serum CPK could also be used to compare fracture series because it could be used to allocate fractures into groups of comparable severity. Furthermore, as pointed out by Larsson and van der Linden (1981), the serum CPK levels could be used as an indicator of impending compartment syndrome. Since the levels begin to fall 3 days after fracture, a continued rise in CPK level could indicate continuing damage to the muscles.

It has not been possible from these results to confirm the claim that trauma precipitates hypo-phosphataemia (Loven et al. 1982) because the pre-accident levels for each patient are not available for comparison. Nevertheless, the serum inorganic phosphate levels change immediately after fracture. The baseline values were not in general lower after high energy violence. The differences between the trauma groups are not statistically significant and, therefore, this is not a good indicator of the severity of trauma as claimed by Gransberg et al. (1971), Lennquist et al. (1979) and by Loven et al. (1982). The clinical relevance and significance of the fluctuations in the serum calcium levels immediately following fractures is not clear. Differences in the serum calcium levels within the trauma and the healing groups may simply reflect what is
happening to the inorganic phosphate levels or vice versa.

The results also show that the serum levels of calcium and inorganic phosphate change during healing. In general, both calcium and inorganic phosphate levels show a more rapid downward trend in delayed compared with normal union following the initial rise in values. Speed (1930) and Urist et al. (1954) observed no changes in the serum levels in their studies. On the other hand, in this study, the phosphate levels were not persistently low in delayed union as claimed by Henderson et al. (1926). In addition, the results have not confirmed the inorganic phosphate fluxes described by Rudd (1926) who found that an initial phase of hypo-phosphataemia coincided with early callus formation and a later phase of hyper-phosphataemia coincided with active calcification. Although these conflicting results may be due to differences in the fracture population between the series, it is probable that phosphate and calcium levels are not sensitive enough to be used to assess healing. On the other hand, the sensitivity may have been affected by the fact that test specimens were usually obtained from non fasting subjects.

The serum creatinine levels were not significantly different between one trauma group and another or between one healing group and the other. This suggests that renal function was similar in these groups and, therefore, the serum inorganic phosphate levels were not unduely influenced by renal function in any of the groups.

It is rather surprising that there was very little
variation in the serum levels of osteocalcin during the course of healing. Theoretical considerations suggest that serum concentrations ought to increase with time as new bone was formed at the fracture site. In the study reported by Price et al. (1980) patients with bone disease characterised by increased bone formation had significantly high levels of osteocalcin. Others, notably Brown et al. (1984), have demonstrated a close correlation between osteoid volume and serum osteocalcin levels. The only positive finding in this study is that the mean osteocalcin level at each interval of measurement was lower in delayed compared with normal union (Table 20). Thus, if serum osteocalcin levels truly reflect bone metabolism, then these results suggest that delayed union may be due to diminished osteoblastic activity. However, it is not clear whether this is due to a decrease in the number of osteoblasts or in their functional integrity. Histological data is not available in these patients and, therefore, it is not possible to determine whether delayed union is a true metabolic bone disease.

It is premature to suggest any role for osteocalcin measurements in the early diagnosis of delayed union, and in making treatment decisions. Nevertheless, it is interesting that differences in osteocalcin levels between the two healing groups were statistically significant at 16 weeks (p=0.0010). Significant differences were also manifest with regards to inorganic phosphate (p=0.020) at the 16th week, and calcium (p=0.053) at the 14th week. Many surgeons consider bone grafting for delayed union between the 12th and the 16th week after fracture. Measurement of biochemical parameters is a non
invasive method of assessing bone formation and this study suggests that it may help the decision making process during this period.

Somatomedin (SM) is the generic name for a family of growth stimulating peptides (growth factors) present in the blood through which some of the effects of growth hormone appear to be mediated (D'Ercole et al. 1980, Spencer 1985). The somatomedins are structurally similar to insulin and are produced in the liver and possibly in the kidney and locally in bone (Stracke et al. 1984). They are anabolic agents which stimulate increases in the number of cells (hyperplasia) and in their sizes (hypertrophy). This study shows that there is no significant difference in SM activity between high and low energy violence. Thus, SM activity is not a good indicator of severity of trauma in closed adult tibial shaft fractures. These results do not confirm the findings by Coates et al. (1981, 1982) of depressed somatomedin production after trauma. This may be due to the fact that these authors used bioassays which may measure a different content of somatomedins (Phillips & Vaassilopoulou-Sellin 1980a & b). Furthermore, it is possible that their patients may have been more severely ill and a number of them died from their injuries.

It is also shown that there is no difference in SM activity during the first 6 weeks after fracture, when proliferative activities at the fracture site are at their most intense, between normal and delayed union. Ashton et al. (1986) came to a similar conclusion. Thus, somatomedin activity is not a good indicator of the healing potential of closed adult
tibial shaft fractures treated by closed methods. This is presumably because not all growth phenomena are under strict pituitary control. According to Urist (1972), for example, mitotic activity and regeneration of the liver following partial hepatectomy is not significantly impaired by hypophysectomy. The animal species used by Hsu and Robinson (1969) in their study was very fragile and consequently, almost half died before conclusion of the experiment. It is possible that delayed union was due to factors other than lack of growth hormone or somatomedins in these animals.
Table 5: Mean early serum CPK values (iu/l) following low and high energy violence.

<table>
<thead>
<tr>
<th></th>
<th>Low energy mean</th>
<th>SD</th>
<th>High energy mean</th>
<th>SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>350.0</td>
<td>217.7</td>
<td>442.2</td>
<td>270.6</td>
<td>-1.14</td>
<td>0.265</td>
</tr>
<tr>
<td>Day 1</td>
<td>369.2</td>
<td>231.8</td>
<td>627.1</td>
<td>304.1</td>
<td>-2.74</td>
<td>0.012*</td>
</tr>
<tr>
<td>Day 2</td>
<td>242.3</td>
<td>167.8</td>
<td>617.4</td>
<td>320.7</td>
<td>-3.83</td>
<td>0.001*</td>
</tr>
<tr>
<td>Day 14</td>
<td>133.1</td>
<td>70.8</td>
<td>109.3</td>
<td>73.8</td>
<td>1.05</td>
<td>0.305</td>
</tr>
</tbody>
</table>

*significant difference

Table 6: Mean early serum inorganic phosphate values (mmol/l) following low and high energy violence.

<table>
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<tr>
<th></th>
<th>Low energy mean</th>
<th>SD</th>
<th>High energy mean</th>
<th>SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>1.02</td>
<td>0.22</td>
<td>0.93</td>
<td>0.26</td>
<td>1.17</td>
<td>0.254</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.05</td>
<td>0.20</td>
<td>1.14</td>
<td>0.18</td>
<td>-1.49</td>
<td>0.150</td>
</tr>
<tr>
<td>Day 2</td>
<td>1.11</td>
<td>0.20</td>
<td>1.12</td>
<td>0.21</td>
<td>-0.19</td>
<td>0.850</td>
</tr>
<tr>
<td>Day 14</td>
<td>1.26</td>
<td>0.19</td>
<td>1.34</td>
<td>0.20</td>
<td>-1.28</td>
<td>0.213</td>
</tr>
</tbody>
</table>
### Table 7: Mean early serum calcium values (mmol/l) following low and high energy violence.

<table>
<thead>
<tr>
<th></th>
<th>Low energy mean</th>
<th>SD</th>
<th>High energy mean</th>
<th>SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>2.26</td>
<td>0.08</td>
<td>2.19</td>
<td>0.10</td>
<td>2.48</td>
<td>0.021*</td>
</tr>
<tr>
<td>Day 1</td>
<td>2.25</td>
<td>0.07</td>
<td>2.19</td>
<td>0.09</td>
<td>2.19</td>
<td>0.042*</td>
</tr>
<tr>
<td>Day 2</td>
<td>2.27</td>
<td>0.09</td>
<td>2.24</td>
<td>0.06</td>
<td>1.04</td>
<td>0.307</td>
</tr>
<tr>
<td>Day 14</td>
<td>2.39</td>
<td>0.08</td>
<td>2.32</td>
<td>0.10</td>
<td>2.29</td>
<td>0.031*</td>
</tr>
</tbody>
</table>

*Significant difference

### Table 8: Mean early total serum alkaline phosphatase values (iu/l) following low and high energy violence.

<table>
<thead>
<tr>
<th></th>
<th>Low energy mean</th>
<th>SD</th>
<th>High energy mean</th>
<th>SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>100.9</td>
<td>45.8</td>
<td>85.4</td>
<td>35.0</td>
<td>1.22</td>
<td>0.233</td>
</tr>
<tr>
<td>Day 1</td>
<td>96.1</td>
<td>46.0</td>
<td>82.4</td>
<td>39.8</td>
<td>0.90</td>
<td>0.378</td>
</tr>
<tr>
<td>Day 2</td>
<td>90.1</td>
<td>36.7</td>
<td>79.4</td>
<td>33.7</td>
<td>0.84</td>
<td>0.410</td>
</tr>
<tr>
<td>Day 14</td>
<td>111.1</td>
<td>38.2</td>
<td>114.5</td>
<td>51.8</td>
<td>-0.22</td>
<td>0.829</td>
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</table>
Table 9: Mean early creatinine values (iu/l) following low and high energy violence.

<table>
<thead>
<tr>
<th></th>
<th>High energy</th>
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<th>Low energy</th>
<th></th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
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<td></td>
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<td>mean SD</td>
<td>t value</td>
<td>p value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>85.4 17.1</td>
<td>84.5 17.2</td>
<td>0.15</td>
<td>0.878</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>87.8 13.9</td>
<td>83.0 12.9</td>
<td>1.05</td>
<td>0.304</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>84.9 12.8</td>
<td>86.3 12.9</td>
<td>-0.32</td>
<td>0.751</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>79.1 12.3</td>
<td>78.4 12.7</td>
<td>0.19</td>
<td>0.849</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 10: Mean early serum CKP values (iu/l) in normal and delayed union.

<table>
<thead>
<tr>
<th></th>
<th>Normal union</th>
<th></th>
<th>Delayed union</th>
<th></th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean SD</td>
<td>mean SD</td>
<td>t value</td>
<td>p value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>390.0 245.3</td>
<td>276.2 162.8</td>
<td>1.57</td>
<td>0.150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>462.4 296.0</td>
<td>475.4 233.5</td>
<td>-0.11</td>
<td>0.914</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>382.8 309.9</td>
<td>514.8 292.8</td>
<td>-0.91</td>
<td>0.398</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>130.4 73.8</td>
<td>113.6 69.8</td>
<td>0.65</td>
<td>0.530</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 11: Mean early serum inorganic phosphate values (mmol/l) in normal and delayed union.

<table>
<thead>
<tr>
<th></th>
<th>Normal union mean</th>
<th>SD</th>
<th>Delayed union mean</th>
<th>SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>1.01</td>
<td>0.23</td>
<td>0.92</td>
<td>0.21</td>
<td>1.14</td>
<td>0.276</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.08</td>
<td>0.21</td>
<td>1.09</td>
<td>0.17</td>
<td>-0.23</td>
<td>0.828</td>
</tr>
<tr>
<td>Day 2</td>
<td>1.14</td>
<td>0.20</td>
<td>1.02</td>
<td>0.20</td>
<td>1.24</td>
<td>0.253</td>
</tr>
<tr>
<td>Day 14</td>
<td>1.29</td>
<td>0.18</td>
<td>1.26</td>
<td>0.25</td>
<td>0.32</td>
<td>0.757</td>
</tr>
</tbody>
</table>

Table 12: Mean early serum calcium values (mmol/l) in normal and delayed union.

<table>
<thead>
<tr>
<th></th>
<th>Normal union mean</th>
<th>SD</th>
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<th>SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>2.25</td>
<td>0.10</td>
<td>2.18</td>
<td>0.06</td>
<td>2.80</td>
<td>0.012*</td>
</tr>
<tr>
<td>Day 1</td>
<td>2.24</td>
<td>0.08</td>
<td>2.21</td>
<td>0.09</td>
<td>0.07</td>
<td>0.507</td>
</tr>
<tr>
<td>Day 2</td>
<td>2.27</td>
<td>0.08</td>
<td>2.20</td>
<td>0.03</td>
<td>3.27</td>
<td>0.005*</td>
</tr>
<tr>
<td>Day 14</td>
<td>2.38</td>
<td>0.09</td>
<td>2.32</td>
<td>0.09</td>
<td>1.55</td>
<td>0.146</td>
</tr>
</tbody>
</table>

*significant difference
### Table 13: Mean early total serum alkaline phosphatase values (iu/l) in normal and delayed union.

<table>
<thead>
<tr>
<th></th>
<th>Normal mean</th>
<th>SD</th>
<th>Delayed mean</th>
<th>SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>95.6</td>
<td>31.6</td>
<td>105.5</td>
<td>84.5</td>
<td>-0.33</td>
<td>0.754</td>
</tr>
<tr>
<td>Day 1</td>
<td>87.8</td>
<td>30.1</td>
<td>110.8</td>
<td>87.3</td>
<td>-0.64</td>
<td>0.550</td>
</tr>
<tr>
<td>Day 2</td>
<td>88.1</td>
<td>36.8</td>
<td>75.0</td>
<td>28.4</td>
<td>0.96</td>
<td>0.363</td>
</tr>
<tr>
<td>Day 14</td>
<td>114.2</td>
<td>42.6</td>
<td>103.1</td>
<td>40.9</td>
<td>0.73</td>
<td>0.480</td>
</tr>
</tbody>
</table>

### Table 14: Mean early serum creatinine values (iu/l) in normal and delayed union.

<table>
<thead>
<tr>
<th></th>
<th>Normal mean</th>
<th>SD</th>
<th>Delayed mean</th>
<th>SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>84.2</td>
<td>16.8</td>
<td>89.0</td>
<td>18.2</td>
<td>-0.68</td>
<td>0.513</td>
</tr>
<tr>
<td>Day 1</td>
<td>86.4</td>
<td>13.7</td>
<td>85.5</td>
<td>14.1</td>
<td>0.14</td>
<td>0.890</td>
</tr>
<tr>
<td>Day 2</td>
<td>85.1</td>
<td>12.2</td>
<td>88.5</td>
<td>13.9</td>
<td>-0.56</td>
<td>0.594</td>
</tr>
<tr>
<td>Day 14</td>
<td>78.8</td>
<td>11.8</td>
<td>79.9</td>
<td>15.2</td>
<td>-0.20</td>
<td>0.847</td>
</tr>
</tbody>
</table>
### Table 15: Mean fortnightly serum CPK values (iu/l) in normal and delayed union.

<table>
<thead>
<tr>
<th></th>
<th>Normal union</th>
<th></th>
<th>Delayed union</th>
<th></th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>SD</td>
<td>mean</td>
<td>SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>410.5</td>
<td>247.9</td>
<td>286.3</td>
<td>187.1</td>
<td>1.44</td>
<td>0.188</td>
</tr>
<tr>
<td>Week 2</td>
<td>118.4</td>
<td>63.6</td>
<td>123.1</td>
<td>68.7</td>
<td>-0.18</td>
<td>0.862</td>
</tr>
<tr>
<td>Week 4</td>
<td>100.7</td>
<td>52.1</td>
<td>98.3</td>
<td>47.0</td>
<td>0.12</td>
<td>0.906</td>
</tr>
<tr>
<td>Week 6</td>
<td>93.0</td>
<td>63.2</td>
<td>91.0</td>
<td>39.1</td>
<td>0.11</td>
<td>0.916</td>
</tr>
<tr>
<td>Week 8</td>
<td>89.6</td>
<td>40.1</td>
<td>97.9</td>
<td>59.2</td>
<td>-0.39</td>
<td>0.706</td>
</tr>
<tr>
<td>Week 10</td>
<td>97.3</td>
<td>45.6</td>
<td>111.7</td>
<td>39.1</td>
<td>-0.80</td>
<td>0.449</td>
</tr>
<tr>
<td>Week 12</td>
<td>90.4</td>
<td>39.8</td>
<td>91.0</td>
<td>41.1</td>
<td>0.07</td>
<td>0.947</td>
</tr>
<tr>
<td>Week 14</td>
<td>105.8</td>
<td>78.6</td>
<td>116.5</td>
<td>53.1</td>
<td>-0.38</td>
<td>0.711</td>
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<tr>
<td>Week 16</td>
<td>91.6</td>
<td>49.0</td>
<td>112.5</td>
<td>41.7</td>
<td>-0.89</td>
<td>0.416</td>
</tr>
<tr>
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<td>90.9</td>
<td>37.3</td>
<td>94.8</td>
<td>33.6</td>
<td>0.20</td>
<td>0.850</td>
</tr>
<tr>
<td>Week 20</td>
<td>88.2</td>
<td>39.5</td>
<td>128.5</td>
<td>17.7</td>
<td>-2.46</td>
<td>0.096</td>
</tr>
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</table>

### Table 16: Mean fortnightly serum inorganic phosphate values (mmol/l) in normal and delayed union.

<table>
<thead>
<tr>
<th></th>
<th>Normal union</th>
<th></th>
<th>Delayed union</th>
<th></th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td>SD</td>
<td>mean</td>
<td>SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>1.01</td>
<td>0.24</td>
<td>0.92</td>
<td>0.21</td>
<td>1.14</td>
<td>0.276</td>
</tr>
<tr>
<td>Week 2</td>
<td>1.28</td>
<td>0.20</td>
<td>1.28</td>
<td>0.21</td>
<td>0.00</td>
<td>1.000</td>
</tr>
<tr>
<td>Week 4</td>
<td>1.29</td>
<td>0.16</td>
<td>1.29</td>
<td>0.19</td>
<td>0.11</td>
<td>0.915</td>
</tr>
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<td>Week 6</td>
<td>1.25</td>
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<td>1.25</td>
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<td>0.938</td>
</tr>
<tr>
<td>Week 8</td>
<td>1.23</td>
<td>0.16</td>
<td>1.19</td>
<td>0.16</td>
<td>0.68</td>
<td>0.507</td>
</tr>
<tr>
<td>Week 10</td>
<td>1.19</td>
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<td>0.15</td>
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</tr>
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<td>1.16</td>
<td>0.14</td>
<td>0.52</td>
<td>0.616</td>
</tr>
<tr>
<td>Week 14</td>
<td>1.16</td>
<td>0.15</td>
<td>1.14</td>
<td>0.09</td>
<td>0.46</td>
<td>0.654</td>
</tr>
<tr>
<td>Week 16</td>
<td>1.25</td>
<td>0.18</td>
<td>1.11</td>
<td>0.07</td>
<td>2.56</td>
<td>0.020*</td>
</tr>
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<td>1.16</td>
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<td>0.10</td>
<td>0.91</td>
<td>0.385</td>
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<td>1.07</td>
<td>0.16</td>
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<td>0.588</td>
</tr>
</tbody>
</table>

*significant difference
### Table 17: Mean fortnightly serum calcium values (mmol/l) in normal and delayed union.

<table>
<thead>
<tr>
<th></th>
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<th>Delayed union mean</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Week 0</strong></td>
<td>2.20 ± 0.35</td>
<td>2.19 ± 0.06</td>
<td>0.17</td>
<td>0.863</td>
</tr>
<tr>
<td><strong>Week 2</strong></td>
<td>2.37 ± 0.09</td>
<td>2.38 ± 0.06</td>
<td>-0.15</td>
<td>0.883</td>
</tr>
<tr>
<td><strong>Week 4</strong></td>
<td>2.36 ± 0.09</td>
<td>2.33 ± 0.06</td>
<td>1.30</td>
<td>0.210</td>
</tr>
<tr>
<td><strong>Week 6</strong></td>
<td>2.34 ± 0.08</td>
<td>2.35 ± 0.11</td>
<td>-0.05</td>
<td>0.961</td>
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<tr>
<td><strong>Week 8</strong></td>
<td>2.36 ± 0.07</td>
<td>2.37 ± 0.04</td>
<td>-0.26</td>
<td>0.797</td>
</tr>
<tr>
<td><strong>Week 10</strong></td>
<td>2.33 ± 0.10</td>
<td>2.35 ± 0.05</td>
<td>-0.67</td>
<td>0.514</td>
</tr>
<tr>
<td><strong>Week 12</strong></td>
<td>2.33 ± 0.09</td>
<td>2.33 ± 0.06</td>
<td>0.13</td>
<td>0.900</td>
</tr>
<tr>
<td><strong>Week 14</strong></td>
<td>2.32 ± 0.07</td>
<td>2.29 ± 0.03</td>
<td>2.04</td>
<td>0.053*</td>
</tr>
<tr>
<td><strong>Week 16</strong></td>
<td>2.34 ± 0.09</td>
<td>2.30 ± 0.06</td>
<td>0.88</td>
<td>0.401</td>
</tr>
<tr>
<td><strong>Week 18</strong></td>
<td>2.31 ± 0.08</td>
<td>2.30 ± 0.08</td>
<td>0.18</td>
<td>0.856</td>
</tr>
<tr>
<td><strong>Week 20</strong></td>
<td>2.34 ± 0.06</td>
<td>2.35 ± 0.08</td>
<td>-0.32</td>
<td>0.773</td>
</tr>
</tbody>
</table>

*Significant difference

### Table 18: Mean fortnightly total serum alkaline phosphatase values (iu/l) in normal and delayed union.

<table>
<thead>
<tr>
<th></th>
<th>Normal union mean</th>
<th>Delayed union mean</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Week 0</strong></td>
<td>94.6 ± 31.4</td>
<td>105.5 ± 84.5</td>
<td>-0.36</td>
<td>0.728</td>
</tr>
<tr>
<td><strong>Week 2</strong></td>
<td>114.0 ± 41.8</td>
<td>102.9 ± 44.1</td>
<td>0.65</td>
<td>0.531</td>
</tr>
<tr>
<td><strong>Week 4</strong></td>
<td>118.1 ± 35.3</td>
<td>111.9 ± 53.3</td>
<td>-0.31</td>
<td>0.762</td>
</tr>
<tr>
<td><strong>Week 6</strong></td>
<td>112.9 ± 29.1</td>
<td>117.3 ± 57.0</td>
<td>-0.20</td>
<td>0.848</td>
</tr>
<tr>
<td><strong>Week 8</strong></td>
<td>109.5 ± 34.0</td>
<td>107.1 ± 46.3</td>
<td>0.14</td>
<td>0.889</td>
</tr>
<tr>
<td><strong>Week 10</strong></td>
<td>110.6 ± 32.7</td>
<td>123.5 ± 54.5</td>
<td>-0.56</td>
<td>0.595</td>
</tr>
<tr>
<td><strong>Week 12</strong></td>
<td>109.6 ± 30.6</td>
<td>121.4 ± 50.9</td>
<td>-0.59</td>
<td>0.572</td>
</tr>
<tr>
<td><strong>Week 14</strong></td>
<td>114.1 ± 29.4</td>
<td>122.8 ± 46.6</td>
<td>-0.44</td>
<td>0.676</td>
</tr>
<tr>
<td><strong>Week 16</strong></td>
<td>104.7 ± 37.4</td>
<td>136.2 ± 51.5</td>
<td>-1.29</td>
<td>0.253</td>
</tr>
<tr>
<td><strong>Week 18</strong></td>
<td>108.4 ± 37.9</td>
<td>107.8 ± 34.1</td>
<td>0.03</td>
<td>0.974</td>
</tr>
<tr>
<td><strong>Week 20</strong></td>
<td>97.1 ± 18.9</td>
<td>113.3 ± 36.2</td>
<td>-0.86</td>
<td>0.444</td>
</tr>
</tbody>
</table>
Table 19: Mean fortnightly serum creatinine values (iu/l) in normal and delayed union.

<table>
<thead>
<tr>
<th>Week</th>
<th>Normal mean</th>
<th>Normal SD</th>
<th>Delayed mean</th>
<th>Delayed SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>83.9</td>
<td>17.0</td>
<td>89.0</td>
<td>18.2</td>
<td>-0.73</td>
<td>0.484</td>
</tr>
<tr>
<td>2</td>
<td>77.8</td>
<td>10.8</td>
<td>80.9</td>
<td>15.0</td>
<td>-0.55</td>
<td>0.593</td>
</tr>
<tr>
<td>4</td>
<td>79.5</td>
<td>13.0</td>
<td>82.3</td>
<td>11.7</td>
<td>-0.59</td>
<td>0.567</td>
</tr>
<tr>
<td>6</td>
<td>79.8</td>
<td>14.3</td>
<td>80.4</td>
<td>13.8</td>
<td>-0.11</td>
<td>0.911</td>
</tr>
<tr>
<td>8</td>
<td>78.4</td>
<td>13.0</td>
<td>78.3</td>
<td>13.0</td>
<td>0.01</td>
<td>0.993</td>
</tr>
<tr>
<td>10</td>
<td>78.5</td>
<td>12.6</td>
<td>79.0</td>
<td>10.7</td>
<td>-0.11</td>
<td>0.916</td>
</tr>
<tr>
<td>12</td>
<td>77.0</td>
<td>14.9</td>
<td>80.9</td>
<td>9.2</td>
<td>-0.89</td>
<td>0.391</td>
</tr>
<tr>
<td>14</td>
<td>77.2</td>
<td>11.6</td>
<td>82.3</td>
<td>13.3</td>
<td>-0.88</td>
<td>0.410</td>
</tr>
<tr>
<td>16</td>
<td>73.7</td>
<td>15.5</td>
<td>78.2</td>
<td>6.5</td>
<td>-0.86</td>
<td>0.407</td>
</tr>
<tr>
<td>18</td>
<td>76.9</td>
<td>13.2</td>
<td>83.0</td>
<td>5.1</td>
<td>-1.55</td>
<td>0.138</td>
</tr>
<tr>
<td>20</td>
<td>75.8</td>
<td>13.5</td>
<td>83.3</td>
<td>4.0</td>
<td>-1.93</td>
<td>0.070</td>
</tr>
</tbody>
</table>

Table 20: Mean serum osteocalcin values (ng/l) in normal and delayed union.

<table>
<thead>
<tr>
<th>Week</th>
<th>Normal mean</th>
<th>Normal SD</th>
<th>Delayed mean</th>
<th>Delayed SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.57</td>
<td>4.37</td>
<td>3.10</td>
<td>1.49</td>
<td>1.97</td>
<td>0.089</td>
</tr>
<tr>
<td>4</td>
<td>7.58</td>
<td>2.92</td>
<td>4.33</td>
<td>2.40</td>
<td>1.99</td>
<td>0.087</td>
</tr>
<tr>
<td>8</td>
<td>6.62</td>
<td>2.93</td>
<td>3.49</td>
<td>1.52</td>
<td>2.36</td>
<td>0.050*</td>
</tr>
<tr>
<td>12</td>
<td>5.41</td>
<td>3.26</td>
<td>4.33</td>
<td>3.59</td>
<td>0.57</td>
<td>0.58</td>
</tr>
<tr>
<td>16</td>
<td>7.49</td>
<td>1.79</td>
<td>3.41</td>
<td>1.64</td>
<td>4.44</td>
<td>0.0010*</td>
</tr>
</tbody>
</table>

*significant difference
Table 21: Mean serum somatomedin values (nmol/l) following low and high energy violence.

<table>
<thead>
<tr>
<th></th>
<th>Low energy mean</th>
<th>Low energy SD</th>
<th>High energy mean</th>
<th>High energy SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>25.08</td>
<td>9.83</td>
<td>19.50</td>
<td>4.54</td>
<td>1.87</td>
<td>0.080</td>
</tr>
<tr>
<td>Week 2</td>
<td>25.00</td>
<td>8.12</td>
<td>19.00</td>
<td>8.98</td>
<td>1.82</td>
<td>0.081</td>
</tr>
<tr>
<td>Week 4</td>
<td>22.20</td>
<td>12.80</td>
<td>22.07</td>
<td>7.52</td>
<td>0.02</td>
<td>0.980</td>
</tr>
<tr>
<td>Week 6</td>
<td>20.62</td>
<td>8.63</td>
<td>18.43</td>
<td>7.31</td>
<td>0.71</td>
<td>0.490</td>
</tr>
</tbody>
</table>

Table 22: Mean serum somatomedin values (nmol/l) in normal and delayed union.

<table>
<thead>
<tr>
<th></th>
<th>Normal union mean</th>
<th>Normal union SD</th>
<th>Delayed union mean</th>
<th>Delayed union SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>22.07</td>
<td>7.18</td>
<td>22.31</td>
<td>8.97</td>
<td>-0.08</td>
<td>0.94</td>
</tr>
<tr>
<td>Week 2</td>
<td>22.57</td>
<td>8.07</td>
<td>21.21</td>
<td>10.1</td>
<td>0.40</td>
<td>0.69</td>
</tr>
<tr>
<td>Week 4</td>
<td>22.5</td>
<td>10.4</td>
<td>21.7</td>
<td>10.3</td>
<td>0.20</td>
<td>0.84</td>
</tr>
<tr>
<td>Week 6</td>
<td>21.93</td>
<td>5.92</td>
<td>16.85</td>
<td>9.09</td>
<td>1.71</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Figure 9  Influence of violence on serum CPK levels (normal values <200 iu/l)

\[ p=0.012 \]
\[ p=0.001 \]

\[ x-x \text{ low energy} \]
\[ (n=17-35) \]

\[ o-o \text{ high energy} \]
\[ (n=12-15) \]

---

Figure 10  Influence of violence on serum inorganic phosphate levels (normal values 0.8-1.4 mmol/l)

\[ x-x \text{ low energy} \]
\[ (n=19-35) \]

\[ o-o \text{ high energy} \]
\[ (n=12-15) \]
Figure 11 Influence of violence on serum calcium levels  
(normal values 2.1–2.6 mmol/l)

Figure 12 Influence of violence on total serum alkaline phosphatase levels  
(normal values = 40–130 iu/l)
Figure 13  Influence of violence on serum creatinine levels  
(normal values 60–120 iu/l)

Figure 14  Early serum CPK levels in normal and delayed union  
(normal values = <200 iu/l)
Figure 15  Early serum inorganic phosphate levels in normal and delayed union (normal values = 0.8 - 1.4 mmol/l)

<table>
<thead>
<tr>
<th>Normal Union (n=26-41)</th>
<th>Delayed Union (n=5-9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>x-x</td>
<td>o-o</td>
</tr>
<tr>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>0.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>

M ± 1SD

Figure 16  Early serum calcium levels in normal and delayed union (normal values = 2.1-2.6 mmol/l)

<table>
<thead>
<tr>
<th>Normal Union (n=26-41)</th>
<th>Delayed Union (n=6-9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>x-x</td>
<td>o-o</td>
</tr>
<tr>
<td>2.4</td>
<td>2.3</td>
</tr>
<tr>
<td>2.3</td>
<td>2.2</td>
</tr>
<tr>
<td>2.2</td>
<td>2.1</td>
</tr>
</tbody>
</table>

M ± 1SD
Figure 17  Early total serum alkaline phosphatase levels in normal and delayed union (normal values 40–130 iu/l)

Figure 18  Early serum creatinine levels in normal and delayed union (normal values 60–120 iu/l)
Figure 19  Sequential serum CPK levels in normal and delayed union
(normal values = <200 iu/l)

Figure 20  Sequential serum inorganic phosphate levels in normal and delayed union
(normal values = 0.8-1.4 mmol/l)
Figure 21  Sequential serum calcium levels in normal and delayed union
(normal values 2.1-2.6 mmol/l)

Figure 22  Sequential total serum alkaline phosphatase levels in normal and delayed union
(normal values = 40-130 iu/l)
Figure 23  Sequential serum creatinine levels in normal and delayed union
(normal values 60-120 iu/l)

Figure 24  Sequential serum osteocalcin levels in normal and delayed union
(normal values = 5.3 ± 1.7 ng/ml)
**Figure 25**  Influence of severity of trauma on mean sequential somatomedin levels (normal values >9.09 nmol/1)

- **x---x** low energy (n=11-14)
- **o---o** high energy (n=11-13)

**Figure 26**  Sequential serum somatomedin levels in normal and delayed union (normal values >9.09 nmol/1)

- **x---x** normal union (n=13-14)
- **o---o** delayed union (n=9-13)
2.3 AN INVESTIGATION OF THE HEALING POTENTIAL OF CLOSED ADULT TIBIAL SHAFT FRACTURES TREATED BY CLOSED METHODS USING SEQUENTIAL DYNAMIC AND STATIC SCINTIGRAPHY.

2.3.1 Introduction

Radiographic abnormalities are slow to develop in slowly healing fractures and, therefore, interest has developed in bone imaging to predict delayed healing using bone-seeking radionuclides and radionuclide labelled bone-seeking substances. Chiewitz and Hevesy (1935) demonstrated selective localisation of $^{32}$P in bone and since then several other radioactive compounds have been investigated. Subramanian and McAfee in 1971 introduced $^{99m}$Tc labeling of bone-seeking phosphate compounds and this made it possible for radionuclide imaging to be carried out widely, cheaply and safely.

The mechanisms by which bone-seeking chemicals concentrate in bone are not known but there are many theories. Labelled calcium and strontium are thought to exchange with stable calcium ions and $^{18}$F probably replaces the hydroxyl ions on the bony crystal lattice (Pendergrass & Castronovo 1977). Inorganic phosphate complexes are thought to be incorporated into the polyphosphate degradation pathways (Bowen & Garnett 1974). By contrast, inorganic diphosphates are believed to be 'chemisorbed' on to the hydroxyapatite crystals of bone (Francis 1969, Jung et al. 1973).
Although the precise mechanisms may vary, it is generally agreed that the bone-seeking agents are taken up in an increased amount in areas of active bone turnover (Galasko 1975, Hughes et al. 1978, Khan et al. 1979, Matin 1979). Fractures accumulate radionuclides (Wendeberg 1961, Paradis & Kelly 1975, Greiff 1981, Hughes 1981) and some workers believe that the amount of radioactivity is related to the amount of new bone formed (Galasko 1975). Consequently, scintigraphic techniques are presumed to be useful in separating normal union from delayed union.

In practice, the value of bone scintigraphy in predicting the healing potential of individual fractures is uncertain because the results of the relevant investigations are conflicting (Illingworth & Schiess 1971, Jacobs et al. 1981, Auchincloss & Watt 1982, Gregg et al. 1983, 1984 & 1986). However, all these reports were based on groups of patients with different fracture types and different management routines. It is possible that bone scintigraphy may be more sensitive in relatively homogeneous groups.

Therefore, a prospective study was conducted to investigate the healing potential of closed adult tibial shaft fractures treated by closed methods. Closed unilateral fractures were selected to limit the number of biological variables between fractures. The objective of the study was to determine once and for all whether radionuclide bone scintigraphy is a useful quantitative method of predicting healing potential.
2.32 Methods

Half of 100 patients, 11 females and 39 males, aged between 16 and 82 years (\( M \pm 1SD = 30.68 \pm 14.36 \)), presenting at the Leicester Royal Infirmary from 1st August, 1985 to 31 July, 1987 with a closed unilateral tibial shaft fracture and treated by closed methods were studied prospectively.

There were 20 transverse fractures which for ease of reference were designated, as in section 2.1, Type I. There were in addition, 12 Type II or spiral fractures, 17 Type III or oblique fractures and 1 Type IV or comminuted supramalleolar fracture. Forty-one fractures united normally and 9 fractures developed delayed union.

As the timing of scintigraphy in the course of fracture healing is critical (Matin 1979), an attempt was made to carry out the scans on three occasions for each patient: as soon as possible after admission and thereafter at 6 and at 12 weeks. These times were chosen because they correspond to specific presumed landmarks during healing. Immediately after fracture there is damage to the blood supply at the fracture site (Ham 1930, Sevitt 1981). Gothman (1961) demonstrated cessation of blood to certain areas shortly after fracture by perfusion techniques. Similar avascular areas have been observed on scintigrams as 'cold spots' by Gregg et al. (1983, 1984, 1986). Several days or weeks after fracture there is reactive hyperaemia at the fracture site which may be associated with a generalised uptake by the fractured bone (Wray & Lynch 1959, Paradis & Kelly 1975, McCarthy & Hughes 1984). Lund et al.
(1978) and Auchincloss and Watt (1982) have suggested that the scintigraphic changes are established by the sixth week. At 12 weeks, the fracture callus is well established and visible radiologically and decisions about grafting for failure to unite are often considered at this time. Furthermore, Jacobs et al. (1981) have demonstrated differences in the rate of uptake between normal and delayed union at 12 weeks.

Method of scintigraphy:

Scintigraphic examination was conducted in two phases using a Siemens large field of view gamma camera (model BB6513) with a low energy all purpose collimator.

1. Dynamic phase - The patient was positioned supine under the gamma camera with his/her legs as close together as possible. Both tibiae, including the fracture site, were included in the field of view. The contralateral normal tibia was shielded with a piece of plaster of Paris cast. 400 megabecquerels of Technetium-99m methylene diphosphonate was injected intravenously at the same time as imaging was commenced. The recordings were carried out on the Nodecrest computer linked to the gamma camera. Twelve consecutive images, each lasting 5 seconds, followed by 28 consecutive images, each lasting 30 seconds, were automatically collected (total data collection time 15 minutes). The matrix size was 64 by 64 pixels. After the dynamic acquisition had finished, a strip of lead was placed over the fracture site, which had previously been marked on the patient’s plaster cast, and another view was acquired over
one minute in order to provide a permanent reference for the location of the fracture site. The camera's inbuilt anatomical marker was not used because it would have produced an artefact during the acquisition of the dynamic data.

2. Static phase - Static images of each leg were acquired on computer 2 - 4 hours after injection of $^{99}$Tc MDP. The fractured tibia was positioned in the centre of the field of view to allow the maximum length to be imaged. The leg and the camera head were rotated in such a way as to prevent any overlap of the images of the tibia and fibula. Where possible both the proximal and distal tibial metaphyses were included within the field of view. If this was not possible the metaphysis nearest to the fracture site was included within the field of view. A 300 second image (matrix size 128 by 128) was acquired. The fracture site was marked using the camera's own inbuilt anatomical marker. Next, the contralateral normal leg was placed in a similar position under the gamma camera but shielded with a piece of plaster of Paris cast. A 300 second image was then acquired as before.

Processing:

All static images were copied onto film and the computer information was stored on floppy discs.

The study contained 4 views as follows:

View 1 - Dynamic phase (both tibias),
View 2 - Dynamic phase image to show fracture site,
View 3 - Static phase of fractured leg, and
View 4 - Static phase of contralateral normal leg.

Analysis of data:

The data obtained was analysed according to all recently reported methods as follows:

1. Dynamic scintigrams - The consecutive dynamic images were examined and four regions of interest (ROIs) were defined (Fig. 27). Region A was drawn to encompass only the area of increased uptake centered on the fracture site. Next, 3 equal rectangular areas of interest were drawn over both tibiae. Site B was drawn over the normal contralateral tibia at a site corresponding to the fracture site; C was drawn over the fractured tibia proximal or distal to the fracture site and site D was drawn over the unfractured tibia at the same level as C. Activity-time curves of dynamic uptake were obtained from these areas of interest. The following information was extracted from the data:

   a) Jacobs' method (Jacobs et al. 1981) - slopes (S₁, S₂ and S₃) were calculated from the activity-time curves (A, B, and C) according to a formula described by Gregg et al. (1984, 1986):

\[
SLOPE = \frac{c_2-c_1}{c_1 \times 75} \times 100 \% \: MIN^{-1}
\]

where c₁ = counts over ROI at 7.5 minutes and c₂ = counts over ROI at 15 minutes.
The means of the differences in the slopes of the activity-time curves designated $S_1 - S_2$, for excess rate of uptake at the fracture site relative to site B, and $S_1 - S_3$, for excess rate of uptake at the fracture site relative to site C, were calculated for normal and delayed union. The values were then plotted separately against time after fracture.

b) Early uptake ratios (Smith et al. 1987) - $A/B$ and $A/C$ were calculated from the summed counts recorded for 510 seconds starting 5 minutes following injection of radionuclide. The means of early uptake ratios for normal and delayed union were calculated and plotted separately against time after fracture.

2. Static scintigrams - The scintigrams were visually inspected for the patterns of uptake of the radioactive tracer and comparisons were made between the fractured and the contralateral normal tibia. The patterns of uptake were classified as, generalised increased uptake, localised increased uptake and "cold spots", i.e. localised areas of absent uptake (Gregg et al. 1983). Regions of interest (Fig. 27) were also drawn as in the dynamic study and from the activity counts for each area the following information was obtained:

a) Uptake ratios (Smith et al. 1987) - $A/B$ and $A/C$ were calculated and the means for normal and delayed union were plotted separately against time.

b) Net counts - $A-B$, for fracture site relative to site B
over the normal tibia, and A-C, for fracture site relative to site C over the same tibia, were calculated and the means for normal and delayed union were plotted separately against time. This was intended to indicate numerically uptake at the fracture site over and above that at other regions of interest.

c) An 'osteogenesis index' was calculated according to the following formula:

\[
\frac{\text{count rate at fracture site (static)}}{\text{count rate at fracture site (dynamic)}}
\]

for normal and for delayed union and plotted separately against time. This was intended to express an absolute index of osteogenic activity at the fracture site. The dynamic phase was assumed to be dependent more on blood flow (Genant et al. 1974) and the static phase was assumed to reflect both blood flow and affinity of new bone for bone-seeking tracer (Galasko 1984).

2.3.3 Results

In practice, precise timing of scintigraphy was not possible, therefore, examinations carried out 1 to 7 days after fracture were designated as time=0 weeks scintigrams, 5 to 7 weeks after fracture as time=6 weeks scintigrams and 11 to 13 weeks after fracture as time=12 weeks scintigrams. At the beginning of the study, a number of patients objected to a third examination partly because of the publicity surrounding the Chernobyl disaster. Table 23 shows the number of patients examined at each interval.
The consecutive dynamic images at time=0 weeks, time=6 weeks and time=12 weeks respectively are shown in Fig. 28. In the time=0 weeks images (Fig. 28a), there was progressive accumulation of radionuclide in both tibiae. There was often little visual difference between the fractured and the intact bone. At the second and third intervals (Figs. 28b & c), uptake in the fractured tibia was more prominent and radionuclide activity was now concentrating progressively at the fracture site.

The static scintigrams at the different time intervals are shown in Fig. 29. Radionuclide accumulation localised at the fracture site could be observed as early as at time=0 weeks when new bone formation had obviously not begun.

Dynamic scintigraphic data:

The activity-time curves for the different time intervals are shown in Fig. 30 and they are similar to those reported by Jacobs et al. (1981). In general, the curves showed an initial rapid rise from zero value and then the rise became more linear. In the time=0 weeks scintigram (Fig. 30a), the fracture site A and the 'control' site C in the same tibia had similar curves. The curves for 'control' sites B and D in the contralateral tibia were similar. At the second interval (Fig. 30b), curves A and C diverged with the higher values of activity being recorded at the fracture site A. At time=12 weeks (Fig. 30c), the rate of uptake at the 'control' site C in the fractured bone was similar to that at each of the regions of interest in the unfractured contralateral tibia. The rate of
uptake at the fracture site A remained at the higher level.

The results obtained from measuring the excess slopes according to the methods of Jacobs et al. (1981) and Gregg et al. (1984, 1986) are summarized in Tables 24 and 25 and depicted graphically in Fig. 31. The data have been grouped according to the time they were obtained after fracture and the $t$ and $p$ values are shown. Table 24 shows that at each time interval, there was no statistically significant difference in the mean values for excess rate of uptake, $S_1 - S_2$, at the fracture site A relative to a similar site B over the opposite tibia. In Table 25, in the first scintigram, there was a statistically significant difference ($p<0.05$) in the mean values, $S_1 - S_3$, comparing the fracture site A with a site C over the same tibia. The corresponding line graphs in Fig. 31 show the mean values with the SD bars and considerable overlap in values was evident at each time interval. Nevertheless, the $S_1 - S_2$ curve for normal union revealed an almost linear increase in values but the curve for delayed union showed a rise from the first to the second scintigram and then a fall from the second to the third scintigram. The $S_1 - S_3$ curve for normal union is somewhat parallel to, but with higher values than, that for delayed union from the second to the third scintigram.

The mean uptake ratios obtained at each visit from the dynamic scintigrams for the two groups of patients are listed in Tables 26 and 27 and shown graphically in Fig. 32. There was no statistically significant difference in the mean values for early uptake $A/B$ ratio at each interval of measurement. There
was a statistically significant difference (p<0.05) in the mean values for the A/C ratios at 6 weeks between normal and delayed union. The general trend of lower values for delayed union confirms similar findings by Smith et al. (1987). However, data obtained in this series could not be separated into normal or delayed union using a cut off value of 1.3 as suggested in their paper. The line graph in Fig. 32 shows the mean values with the SD bars and considerable overlap in values was evident at each interval. It is interesting to note that there was a progressive increase in uptake ratios which showed a peak at 6 weeks and thereafter declined slightly.

**Static scintigraphic data:**

The findings on static scintigrams at the two earlier time intervals are summarized in Tables 28, 29, 30 and 31. In the normal union group at time=0 weeks (Table 28), there was a generalised increased uptake throughout the whole tibia in 34 of 41 fractures. Five fractures had uptake more localised to the fracture site and definite 'cold spots' were observed in 2 fractures. At time=6 weeks, only 4 of 38 fractures had generalised uptake and 34 had localised uptake. 'Cold spots' were not observed. In the delayed union group at time=0 weeks (Table 29), there was generalised uptake in 6 of 9 fractures, 2 fractures had localised uptake and definite 'cold spots' were observed in one case. At the second scintigraphic interval, uptake was localised to the fracture site in all cases. A similar scintigraphic trend was observed when the fractures were classified according to radiological morphology. Radionuclide uptake was predominantly generalised in the first
scintigrams in all cases and predominantly localised to the fracture site in the second scintigrams in all cases (Tables 30 and 31).

The mean uptake ratios obtained at each visit for the static scintigrams for the two groups of patients are listed in Tables 32 and 33 together with their t and p values. The corresponding line graphs are shown in Fig. 33. As expected from other reports, there was a general trend for delayed union to have lower values. There was no statistically significant differences in the mean values for late uptake A/B ratio at each interval of measurement (Table 32). There was a statistically significant difference (p<0.05) in the A/C ratios at 6 weeks between normal and delayed union. The cut off value in this series was 2.0 with the maximum value for delayed union being 2.09 and the minimum value for normal union being 2.14 (Table 33). The line graph in Fig. 33b shows the values with their SD bars, and significantly, there is no overlap in the mean A/C values for normal and delayed union at the 6 week interval.

The results obtained from calculating the mean net counts at the fracture site are summarized in Tables 34 and 35 and depicted graphically in Fig. 34. There was a statistically significant difference (p<0.05) in the mean values A-B and A-C for normal and delayed union at the 6 week interval. In the corresponding line graphs in Fig. 34, the curves for delayed union showed a linear increase from the time=0 weeks interval. By contrast, the curve for normal union showed an initial rise from time=0 weeks to time=6 weeks and a progressive decline
from time=6 weeks to time=12 weeks. Similar observations have been made by Greiff (1981) and by Hughes (1984).

The relationship between the osteogenesis index and type of union is shown in Table 36. There was a progressive increase in values from the baselines at time=0 weeks. The higher values were recorded for normal union and there was a statistically significant difference (p<0.05) at 6 weeks but this was negated by the considerable overlap in values. Fig. 35 depicts the results graphically.

2.34 Discussion and conclusions

It was not possible in this study to classify individual fractures into normal or delayed union by visual assessment of static images as suggested by Auchincloss and Watt (1982). The results confirm the finding by Gregg et al. (1983, 1984, 1986) of all observed patterns of tracer uptake being represented in both clinical groups. This absence of a relationship between scintigraphic type and ability to heal on time suggests that radionuclide accumulation may not be a specific indicator of new bone formation. In an experimental study with rats, Genant et al. (1974) also showed that radionuclide uptake was independent of the rate of osteogenesis.

Nevertheless, one of the indices of uptake measured in this study appeared to clearly separate normal from delayed union; the A/C ratios obtained from static scintigrams at 6 weeks with a cut off value of 2.0. The early uptake ratio at the first visit did not demonstrate an absolute separation
between normal and delayed union as observed by Smith et al. (1987) in their series but their first scintigrams were carried out at between 2 and 4 weeks after fracture. These uptake ratios represent local radionuclide accumulation at the fracture site and may, therefore, indirectly reflect healing if it is assumed that increased uptake represents osteogenic activity. It is noteworthy that the mean net counts at the fracture site A relative to regions of interest B and C (A-B and A-C) and the osteogenesis index were also markedly different between normal and delayed union at 6 weeks.

On the other hand, uptake ratios may reflect the efficiency of the fracture site in extracting minerals from the blood circulation. If this is so, then in this series, radionuclide extraction at the fracture site may be said to be similar in normal and delayed union shortly after fracture and 12 weeks later but that normally uniting fractures were more efficient at 6 weeks compared with slowly uniting ones. It is significant that the fracture callus often begins to be radiologically apparent at 6 weeks. However, further experimental studies are required to determine whether or not the 6th week is a significant biological landmark in the healing of adult human tibial shaft fractures.

The activity-time curves in the dynamic phase obtained in this study were qualitatively similar to that reported by others (Jacobs et al. 1981, Gregg et al. 1984, 1986). However, the trend of the excess slopes in the present series appears to be different. The excess rate of uptake at the fracture site relative to a second site over the same tibia, S1-S3, revealed
a continuous upward trend for normal union and a biphasic curve with an initial fall in values and then a rise after 6 weeks for delayed union. By contrast, the excess rate of uptake at the fracture site relative to a corresponding site in the contralateral tibia, S1-S2, was biphasic for both healing groups. There was an initial rise and then a fall after 6 weeks. However, there was considerable overlap in values between the groups which suggests that these trends are not significantly different for normally uniting and slowly uniting fractures. Thus, analysis of the rate of uptake as proposed by Jacobs et al. (1979) may not be sensitive enough to identify a fracture as normal or slow healing. Gregg et al. (1984, 1986) used this technique and came to the same conclusions.

Furthermore, during the course of this study the exact siting and sizing of the regions of interest were altered in an attempt to improve the reliability of the method but no change in the overall results were obtained. Thus, the Jacobs/Gregg method of processing the dynamic data cannot be unduly influenced by the investigator.

It is difficult, from these results, to escape the conclusion that the routine use of bone scintigraphy in this fracture may not be entirely straightforward. It would appear that only the A/C ratio is of potential use but its significant numerical value of 2.0 obtained at this centre is different to that reported elsewhere (Smith et al. 1987). Therefore, each centre hoping to use this technique routinely would have to determine their own reference A/C value. This reference value was also obtained at a different time interval after fracture
in this study. This is hardly surprising because the time-scale definition of delayed union is an arbitrary one. It is not related to any known biological landmarks. In this series, only 4 fractures classified as delayed union, and whose attendants felt clinically were not progressing at all, required operative intervention, the rest proceeded to union without interference. Therefore, it may be argued, that most, if not all, of these closed tibial shaft fractures possess the potential to heal but that the time scale for the expression of this is different for individual fractures. Thus, the differences between normal and delayed union may be essentially a quantitative and not a qualitative one.

The results confirm the conclusions reached by Gregg et al. (1986) and by Smith et al. (1987) that the contralateral normal tibia does not provide a useful control site. No prediction could be made from any of the several indices measured with regards to the speed of healing in individual cases when the normal tibia was used as control. This implies that the changes in the blood circulation in the injured limb occur independently of any circulatory changes that may be occurring simultaneously in the uninjured limb.
Table 23: Number of patients examined at each time interval.

<table>
<thead>
<tr>
<th></th>
<th>Normal union</th>
<th>Delayed union</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>time=0</td>
<td>41</td>
<td>9</td>
<td>50</td>
</tr>
<tr>
<td>time=6</td>
<td>38</td>
<td>7</td>
<td>45</td>
</tr>
<tr>
<td>time=12</td>
<td>16</td>
<td>4</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 24: Sequential dynamic scintigrams - mean excess slopes of activity-time curves $S_1 - S_2$.

<table>
<thead>
<tr>
<th></th>
<th>Normal Union Mean</th>
<th>SD</th>
<th>Delayed Union Mean</th>
<th>SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>time=0</td>
<td>-0.025</td>
<td>0.794</td>
<td>0.77</td>
<td>1.01</td>
<td>-2.07</td>
<td>0.068</td>
</tr>
<tr>
<td>time=6</td>
<td>2.02</td>
<td>1.38</td>
<td>2.440</td>
<td>0.744</td>
<td>-1.03</td>
<td>0.33</td>
</tr>
<tr>
<td>time=12</td>
<td>1.92</td>
<td>1.48</td>
<td>1.767</td>
<td>0.493</td>
<td>0.34</td>
<td>0.74</td>
</tr>
</tbody>
</table>
Table 25: Sequential dynamic scintigrams - mean excess slopes of activity-time curves S1 - S3.

<table>
<thead>
<tr>
<th></th>
<th>Normal Mean</th>
<th>Delayed Mean</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>time=0</td>
<td>0.09</td>
<td>0.962</td>
<td>-2.70</td>
<td>0.014*</td>
</tr>
<tr>
<td>time=6</td>
<td>1.100</td>
<td>0.380</td>
<td>2.13</td>
<td>0.077</td>
</tr>
<tr>
<td>time=12</td>
<td>1.54</td>
<td>1.075</td>
<td>0.96</td>
<td>0.37</td>
</tr>
</tbody>
</table>

*significant difference

Table 26: Sequential dynamic scintigrams - mean early uptake A/B ratios.

<table>
<thead>
<tr>
<th></th>
<th>Normal Mean</th>
<th>Delayed Mean</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>time=0</td>
<td>2.025</td>
<td>1.900</td>
<td>0.62</td>
<td>0.55</td>
</tr>
<tr>
<td>time=6</td>
<td>3.63</td>
<td>2.680</td>
<td>1.40</td>
<td>0.18</td>
</tr>
<tr>
<td>time=12</td>
<td>3.39</td>
<td>2.77</td>
<td>0.74</td>
<td>0.48</td>
</tr>
</tbody>
</table>
Table 27: Sequential dynamic scintigrams - mean early uptake A/C ratios.

<table>
<thead>
<tr>
<th>Time</th>
<th>Normal Union Mean</th>
<th>Normal Union SD</th>
<th>Delayed Union Mean</th>
<th>Delayed Union SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.072</td>
<td>0.161</td>
<td>0.975</td>
<td>0.116</td>
<td>1.93</td>
<td>0.074</td>
</tr>
<tr>
<td>6</td>
<td>1.822</td>
<td>0.456</td>
<td>1.420</td>
<td>0.205</td>
<td>3.39</td>
<td>0.0069*</td>
</tr>
<tr>
<td>12</td>
<td>1.713</td>
<td>0.362</td>
<td>1.467</td>
<td>0.577</td>
<td>0.71</td>
<td>0.55</td>
</tr>
</tbody>
</table>

*significant difference

Table 28: Distribution of static scintigrams in normal union.

<table>
<thead>
<tr>
<th>Time</th>
<th>Generalised uptake</th>
<th>Localised uptake</th>
<th>'Cold spots'</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>time=0</td>
<td>34 82.9</td>
<td>5 12.2</td>
<td>2 4.9</td>
<td>41</td>
</tr>
<tr>
<td>time=6</td>
<td>4 10.5</td>
<td>34 89.5</td>
<td>-</td>
<td>38</td>
</tr>
</tbody>
</table>
Table 29: Distribution of static scintigrams in delayed union.

<table>
<thead>
<tr>
<th></th>
<th>time=0 No</th>
<th></th>
<th>time=6 No</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td></td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Generalised</td>
<td>7 77.8</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>uptake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localised</td>
<td>2 22.2</td>
<td>7</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>uptake</td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>'Cold spots'</td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9 100.0</td>
<td></td>
<td>7 100.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 30: Influence of radiological fracture morphology on static scintigrams in normal union.

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>time=0 'Cold spots'</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>time=6 'Cold spots'</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>time=0 Local. uptake</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>time=6 Local. uptake</td>
<td>16</td>
<td>8</td>
<td>10</td>
<td>-</td>
<td>34</td>
</tr>
<tr>
<td>time=0 Gen. uptake</td>
<td>14</td>
<td>9</td>
<td>10</td>
<td>1</td>
<td>34</td>
</tr>
<tr>
<td>time=6 Gen. uptake</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>
### Table 31: Influence of radiological fracture morphology on static scintigrams in delayed union.

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Cold spots'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>time=0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>time=6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Local. uptake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>time=0</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>time=6</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Gen. uptake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>time=0</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>time=6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 32: Sequential static scintigrams - mean late uptake A/B ratios.

<table>
<thead>
<tr>
<th></th>
<th>Normal Mean</th>
<th>Normal SD</th>
<th>Delayed Mean</th>
<th>Delayed SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>time=0</td>
<td>2.713</td>
<td>0.876</td>
<td>2.813</td>
<td>0.582</td>
<td>0.39</td>
<td>0.70</td>
</tr>
<tr>
<td>time=6</td>
<td>8.56</td>
<td>3.87</td>
<td>7.72</td>
<td>3.19</td>
<td>0.53</td>
<td>0.62</td>
</tr>
<tr>
<td>time=12</td>
<td>8.22</td>
<td>4.26</td>
<td>9.70</td>
<td>3.08</td>
<td>-0.71</td>
<td>0.53</td>
</tr>
</tbody>
</table>
Table 33: Sequential static scintigrams - mean late uptake A/C ratios.

<table>
<thead>
<tr>
<th></th>
<th>Normal Union</th>
<th>Delayed Union</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>time=0</td>
<td>1.281 0.375</td>
<td>1.25 0.183</td>
<td>1.68</td>
<td>0.11</td>
</tr>
<tr>
<td>time=6</td>
<td>3.40 1.26</td>
<td>1.780 0.311</td>
<td>6.32</td>
<td>0.0000*</td>
</tr>
<tr>
<td>time=12</td>
<td>3.57 1.63</td>
<td>3.33 1.22</td>
<td>0.29</td>
<td>0.79</td>
</tr>
</tbody>
</table>

*significant difference

Table 34: Sequential static scintigrams - mean net count at fracture site A - B.

<table>
<thead>
<tr>
<th></th>
<th>Normal Union</th>
<th>Delayed Union</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>time=0</td>
<td>30.4 24.4</td>
<td>30.25 9.85</td>
<td>0.02</td>
<td>0.98</td>
</tr>
<tr>
<td>time=6</td>
<td>189.0 107.0</td>
<td>116.1 38.3</td>
<td>2.91</td>
<td>0.011*</td>
</tr>
<tr>
<td>time=12</td>
<td>149.4 50.5</td>
<td>190.6 72.5</td>
<td>-0.78</td>
<td>0.58</td>
</tr>
</tbody>
</table>

*significant difference
Table 35: Sequential static scintigrams - mean net count at fracture site A - C.

<table>
<thead>
<tr>
<th></th>
<th>Normal Union Mean</th>
<th>Normal Union SD</th>
<th>Delayed Union Mean</th>
<th>Delayed Union SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>time=0</td>
<td>11.1</td>
<td>16.5</td>
<td>3.92</td>
<td>7.50</td>
<td>1.83</td>
<td>0.079</td>
</tr>
<tr>
<td>time=6</td>
<td>114.5</td>
<td>84.6</td>
<td>58.3</td>
<td>21.6</td>
<td>4.95</td>
<td>0.0000*</td>
</tr>
<tr>
<td>time=12</td>
<td>119.1</td>
<td>42.3</td>
<td>112.9</td>
<td>89.0</td>
<td>0.12</td>
<td>0.92</td>
</tr>
</tbody>
</table>

*significant difference

Table 36: Osteogenesis index for normal and delayed union.

<table>
<thead>
<tr>
<th></th>
<th>Normal Union Mean</th>
<th>Normal Union SD</th>
<th>Delayed Union Mean</th>
<th>Delayed Union SD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>time=0</td>
<td>0.759</td>
<td>0.308</td>
<td>0.700</td>
<td>0.200</td>
<td>0.67</td>
<td>0.52</td>
</tr>
<tr>
<td>time=6</td>
<td>1.587</td>
<td>0.480</td>
<td>1.240</td>
<td>0.279</td>
<td>2.30</td>
<td>0.050*</td>
</tr>
<tr>
<td>time=12</td>
<td>1.850</td>
<td>0.369</td>
<td>1.700</td>
<td>0.361</td>
<td>0.63</td>
<td>0.57</td>
</tr>
</tbody>
</table>

*significant difference
Figure 27  Regions of interest in bone scans
Figure 28a
Sequence of dynamic images at time=0 weeks obtained at approximately 5 second intervals - top left 5 seconds and bottom right 2 minutes - after injection of 99mTc MDP. The site of fracture is indicated by the arrow.

Figure 28b
Sequence of dynamic images at time=6 weeks obtained at approximately 5 second intervals - top left 5 seconds and bottom right 2 minutes - after injection of 99mTc MDP. The site of fracture is indicated by the arrow.
Figure 28c
Sequence of dynamic images at time=12 weeks obtained at approximately 5 second intervals - top left 5 seconds and bottom right 2 minutes - after injection of 99mTc-MDP. The site of fracture is indicated by the arrow.

Figure 29
Sequence of static images at time=0 weeks (top), time=6 weeks (middle), and at time=12 weeks (bottom). The site of fracture is indicated by the arrow.
Figure 30a
Activity-time curves (right) obtained from the dynamic scintigrams (left) at time=0 weeks. The regions of interest are boxed and the site of fracture is indicated by the arrow.

Figure 30b
Activity-time curves (right) obtained from the dynamic scintigrams (left) at time=6 weeks. The regions of interest are boxed and the site of fracture is indicated by the arrow.
Figure 30c
Activity-time curves (right) obtained from the dynamic scintigrams (left) at time=12 weeks. The regions of interest are boxed and the site of fracture is indicated by the arrow.
Figure 31 Relationship between excess slopes of activity-time curves and time after fracture for normal and delayed union a) SI-S2 and b) SI-S3 (± 1SD)
Figure 32  Relationship between early uptake ratios and time after fracture in normal and delayed union a) A/B and b) A/C (M ± 1SD)

- * normal union
- o delayed union

p=0.0069
Figure 33  Relationship between late uptake ratios and time after fracture in normal and delayed union  a) A/B and b) A/C  (M ± 1SD)

- * normal union
- o delayed union

Late uptake ratios

a) A/B

b) A/C

p=0.000
Figure 34  Relationship between net count at fracture site and time after fracture in normal and delayed union a) A-B and b) A-C (M ± 1SD)
Figure 35  Influence of time on the osteogenesis index in normal and delayed union (M ± 1SD)

* normal union
° dynamic union

p=0.050

static/dynamic

weeks
2.4 SUMMARY

A prospective study has been undertaken of 100 closed adult tibial shaft fractures treated by closed methods at the Leicester Royal Infirmary between 1 August, 1985 and 31 July, 1987. The fractures were assessed periodically during the course of healing using clinical, biochemical and scintigraphic methods in an attempt to identify slowly healing fractures. Nineteen per cent of the fractures did not heal at 20 weeks but of these, 15 were united at 30 weeks. Thus, surgical intervention is seldom necessary in these cases. Of all the clinical parameters often suspected of leading to delayed healing, severity of trauma appears to be the most deleterious to rapid healing.

Creatinine phosphokinase (CPK), an enzyme present in skeletal muscles, enters the blood circulation shortly after fracture. The serum levels of CPK increase progressively over several hours after fracture but return to normal after 3 days and correlate well with the initial severity of trauma (as judged clinically). Serum levels of calcium and inorganic phosphate rise from baseline values to peak two weeks after fracture and, thereafter, progressively decline during the entire healing period. The peak calcium values are lower and the peak phosphate levels are higher after high energy violence and the decline in values occurs more rapidly in delayed union. Serum levels of osteocalcin, a non collagenous bone protein, are lower in patients with delayed union particularly at 16 weeks when significant differences are also present in the
calcium and inorganic phosphate levels. Serum levels of somatomedins, a growth stimulating peptide related to insulin and to growth hormone, are not influenced by severity of trauma and are within normal limits in normally healing and slowly healing fractures.

Analysis of the results obtained using bone scintigraphy show that, although there are significant differences between the A/C ratios, mean net counts and osteogenesis index for the group as a whole, the overlap in values means that the method is not practical in individual cases. Only the A/C ratio from the static scintigram at 6 weeks with a cut off value of 2.0 clearly separated normal from delayed union. This value is different from that obtained elsewhere and if it is to be used widely, each centre will have to determine its own reference value.
3.0 EXPERIMENTAL STUDIES OF SOFT TISSUE DAMAGE FOLLOWING FRACTURE
3.1 AN INVESTIGATION OF THE FRACTURE CHARACTERISTICS OF THE MATURE RABBIT TIBIAL DIAPHYSIS.

3.11 Introduction

A study of the fracture characteristics of a material is of major interest to engineers because it provides some indication of strength and toughness (Case & Chilver 1959, Gordon 1968). The mechanism of fracture or relationship between applied force and fracture type (Perkins 1958, Alms 1961) may also be directly observed. This is of some importance to orthopaedic surgeons because it allows an inference to be made about whether a deforming force causing a particular fracture has been directly or indirectly applied (Bauer et al. 1962, Allum & Howbray 1980). The location of associated soft tissue injuries, particularly in closed fractures, may be inferred from the mechanism of fracture (Alms 1961) and according to Charnley (1970) this knowledge is useful in the closed reduction of fractures.

This knowledge may also be important to our understanding of fracture healing because the mechanism of fracture may determine how much energy is dissipated into the soft tissues. This excess energy could cause additional damage depending partly upon whether a material is brittle or ductile (Gordon 1968). In brittle materials, fracture is fast and, therefore, there is significant excess energy available to dissipate into the soft tissues. By contrast, in ductile materials, fracture is slow and more of the elastic energy is
absorbed in the material itself.

Surprisingly, in spite of a voluminous literature on fracture healing, the relationship between the mechanism of fracture and soft tissue damage has not been directly studied. In this study, the fracture characteristics of the tibia of the mature rabbit have been examined. The results have been used to determine the relationship between applied force, fracture type, energy of fracture and soft tissue damage.

3.12 Methods

12 mature male New Zealand white rabbits whose tibiae had stopped growing, that is, weighing more than 3.5kg and aged more than 20 weeks (Masoud et al. 1986a & b), were obtained shortly after death by pentobarbitone overdose. The animals were divided into four biomechanical groups of 3 animals in each group. Fractures of the tibia were produced by an angulatory force in group A, by a spiral force in group B, by compressive force in group C and by combined angulatory and compressive forces in group D. Angulatory and spiral forces were applied manually using padded clamps (Fig. 36) and compressive forces were applied by axial loading applied mechanically to the tibial plateau in the flexed knee. The fractured tibiae were radiographed using a standard medical X-ray machine (at 60kV, 55mA, 80cm FSD and 0.4 sec. exposure time). Trimax screened double emulsion films were used and they were hand developed at room temperature. These X-ray films provided record of the type of fracture produced.
In a parallel study carried out under the direction of the Materials Science Laboratory, Faculty of Engineering, University of Leicester, 6 mature male New Zealand white rabbits were obtained shortly after death by pentobarbitone overdose. The legs were amputated at the knees and the feet were removed. The specimens were divided into 2 groups of 6 legs each. In group I the subcutaneous border of the middle third of the tibia was exposed through a longitudinal incision and a strain gauge, which was connected to a recording apparatus, was fastened directly on to the tibia. The tibia was then subjected to a bending force applied manually through padded clamps, which were attached near each end, until the bone fractured. The strain curve was graphically recorded in order to compare the pattern with the fractures to be produced mechanically.

In group II, 3 tibiae were fitted with strain gauges as above (Fig. 37) and each tibia was then mechanically subjected to a bending stress in a Tensometer and the stress curve was graphically recorded. The remaining 3 tibiae were subjected to torsion stress and the amount of force required to fracture the tibia was directly measured.

In group I, fracture by manual application of force, only bending force could be measured for technical reasons. The strain gauge and the apparatus available could not accurately measure torsional force applied manually. In group II, mechanical application of force, torsional force, unlike bending, was directly measured because the whole tibia could not fit into the apparatus available for automatic recording.
3.13 Results

The epiphysis of the ilium was closed in all animals thus confirming their adult age. None of the tibiae studied showed bowing and/or greenstick or buckle fracture characteristic of immature human bones (Borden 1974, Currey & Butler 1975). The bones were all brittle and gave suddenly in each instance.

Manually applied deforming forces produced a spectrum of fractures (Fig. 38) which were directly related to the nature of the applied force. Angulatory forces (group A) produced 2 oblique fractures, 1 simple transverse fracture and 3 comminuted transverse fractures. Spiral forces (group B) produced 2 simple spiral fractures and 4 comminuted spiral fractures. Compressive forces (group C) did not fracture the shaft but when combined with angulatory forces (group D), comminuted transverse fractures were produced in all cases.

The mechanically fractured bones did not show plastic deformation before fracture and they appeared to fracture in tension mode. Bending stress produced fractures which propagated (Frost 1960) transversely across the width of the bone while torsional stress produced fractures which propagated along the longitudinal axis of the bone. The strain-time curve obtained during manual fracture is shown in Fig. 39 and it is similar to the force-strain curve obtained during mechanical fracture by a bending stress shown in Fig. 40 in that fracture occurred suddenly and with approximately
the same strain value.

The mean value of 3 tests for mechanically applied bending force was 250N and the average moment arm was 16mm (Table 37).

\[ \text{bending moment (M)} = 250 \times 16 \]
\[ = 4000 \text{ Nmm} \]

The mean value of 3 tests for mechanically applied torsion force was 11N and the average moment arm was 170mm (Table 38).

\[ \text{shear moment (T)} = 11 \times 170 \]
\[ = 1870 \text{ Nmm} \]
\[ \text{:: T is approximately half of M.} \]

3.14 Discussion and conclusions

The results of fracture by mechanical methods provide an interesting comparison between fractures produced by bending, that is, transverse/oblique fractures and fractures produced by torsion, that is, spiral fractures. For example, according to the calculations above in the circumstances where the moment arms are equal, a spiral fracture will be caused by a force that is half the magnitude of one that will cause a transverse fracture. This is in line with theoretical predictions of failure criteria for brittle materials which propose that:
maximum bending stress = maximum torsion stress (Case & Chilver 1959)

Maximum bending stress \( (r_b) = \frac{M \cdot y}{I} \)

Maximum torsion stress \( (r_t) = \frac{T \cdot r}{J} \)

\[ \therefore \frac{M \cdot y}{I} = \frac{T \cdot r}{J} \]

where \( M \) = bending moment, \( y \) = radius of bone, \( I \) = bending constant, \( T \) = torsion moment, \( r \) = radius of bone and \( J \) = torsion constant.

as \( y = r \)
for a circular section, \( I = 2J \)
\[ \therefore M = 2T \]

However, this mathematical relationship holds true only if the maximum tensile strength is the same in both cases.

The bending moment was calculated above to be 4000 Nmm and shear moment was 1870 Nmm,

\[ \therefore r_b = \frac{M \cdot y}{I} = 4000 \times \frac{3}{63.5} \]
\[ = 18.9 \, \text{N/mm}^2 \]

and \( r_t = \frac{T \cdot r}{J} = 1870 \times \frac{3.5}{236} \)
\[ = 20.39 \, \text{N/mm}^2 \]

These values for maximum tensile stress are remarkably similar considering the wide natural variations inherent in biological materials. Thus, the prediction concerning the relative magnitude of forces required to cause
transverse/oblique and spiral fractures respectively holds true.

A separate question concerns the amount of energy stored per unit length of bone (Gordon 1968).

\[
\begin{align*}
\text{Bending energy (Em)} &= \frac{M^2}{EI} \\
\text{Torsional energy (Et)} &= \frac{T^2}{EJ} \\
\frac{Em}{Et} &= \frac{M^2 J}{T^2 I} \\
\text{since } M &= 2T \text{ and } I = 2J \text{ (see above),} \\
\text{then } Em &= 2Et
\end{align*}
\]

where \( E \) = Young’s modulus, \( I \) = bending constant, \( J \) = torsion constant, \( M \) = bending moment and \( T \) = torsion moment.

Thus, if a known amount of energy is applied to a bone to cause either a transverse or a spiral fracture, the excess energy remaining after fracture which is available to dissipate in the soft tissues, will be greater for the transverse fracture. Part of this energy may be translated into transverse motion and/or vibrations at the site of transverse/oblique fractures. By contrast, in the case of spiral fractures motion and/or vibrations will take place in the longitudinal axis of the bone. This explains the generally held belief in clinical practice that transverse fractures are in general associated with more serious soft tissue damage than spiral fractures.

All the fractures produced in this study have their clinical equivalents (Perkins 1958, Alms 1961, Allum & Howbray
The fracture characteristics of the mature rabbit tibia appears to be similar to that of adult human bone. Therefore, the mature rabbit tibia is a suitable model for investigating the patterns of soft tissue damage associated with different types of fractures in the adult human bone.

It is interesting to note that the bones of these mature animals do not share the same healing properties as adult human bones as has been remarked by Ham & Harris (1971). This suggests that there is no direct relationship between material properties and biological behaviour with regards to healing. This is presumably because mechanical behaviour of bone depends on osteonal arrangements (Cohen & Harris 1958, Jowsey 1966, Reilly & Burstein 1974) and on physical factors such as Young's modulus, energy-absorbing capacity and density (Evans 1961) while the speed of healing may depend simply on the availability of osteogenic cells.

For instance, the periosteum of the mature rabbit tibia is a relatively thick structure and it is widely believed that this contributes to the rapidity of repair in these animals (Ham & Harris 1971). However, delayed union and non union of tibial shaft fractures occur in early adulthood in the human when the periosteum is also relatively thick. Therefore, the rapidity of healing in rabbits may not be due simply to the thickness of the periosteum but also to other additional factors. For instance, the proximal tibial growth plate was present in all animals. This may be important because accelerated growth, which is a common feature of fracture healing in young patients, has been shown to be due to growth
plate activity (Clement & Colton 1986). In addition, Owen and her colleagues (1955) have identified nests of epiphyseal cartilage in the cortex of mature rabbits. It is conceivable that these cartilage remnants may become activated by a fracture in their vicinity and thereby encourage rapid healing. Prasad and Reynolds (1968) have provided evidence to support this view. They removed the epiphyseal plates from embryo long bones and demonstrated that the diaphysis by itself was capable of longitudinal growth.
**Table 37: Measurements from bending tests.**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Force (N)</th>
<th>Moment arm (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>246</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>249</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>255</td>
<td>13</td>
</tr>
</tbody>
</table>

**Table 38: Measurements from torsion tests.**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Force (N)</th>
<th>Moment arm (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>170</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>180</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>160</td>
</tr>
</tbody>
</table>
Figure 36 Photograph showing manual application of a spiral force

Figure 37 Photograph showing an amputated rabbit leg (1) held in the clamp device (2) which was inserted into the tensometer. Strain gauge applied to anterior aspect of tibia is marked (3)
Figure 38  Radiographs of a spectrum of manually produced fractures showing from left to right; comminuted transverse fracture, oblique fracture and spiral fracture
Figure 39 Strain-time curve for manual fracture by bending

Figure 40 Force-strain curve for mechanical fracture by bending
3.2 AN INVESTIGATION OF THE SOFT TISSUE DAMAGE (PERIOSTEAL, MARROW AND VASCULAR) FOLLOWING EXPERIMENTAL FRACTURES OF THE MATURE RABBIT TIBIAL DIAPHYSIS.

3.21 Introduction

Clinical experience suggests that the speed of healing may depend on the extent of soft tissue damage (Ellis 1958a, Bauer et al. 1962, Wilson 1981, Leach 1984) and on the type of bony damage (Urist et al. 1954). However, neither the bony nor the soft tissue damage is due entirely to the fracture process alone. Concurrent vascular damage may deprive a significant length of each fractured bone end of a blood supply (Ham 1930, Ham & Cormack 1979, Sevitt 1981). Haematoma formation, oedema and inflammatory exudates may interfere with local blood supply or reduce blood flow to the surrounding soft tissues.

Surprisingly, no true information exists about the damage caused directly by a shaft fracture as distinct from other secondary events. Most relevant studies have been conducted in live animals and some time after the fracture had occurred. Damage observed under such circumstances includes additional damage caused by the pathophysiological responses to fracture and to other subsequent events. It is important to our understanding of delayed union that the primary effects of fracture be separated from its secondary effects. For through this knowledge, it may be possible to influence directly the course of healing by preventing deleterious secondary damage.
It is particularly difficult to investigate the soft tissue damage that may accompany shaft fractures. Surgeons presumably have the opportunity to observe periosteal damage during operations for internal fixation of fractures. However, because these are seldom carried out immediately after fracture, most surgeons will be observing additional effects of inflammation, tissue oedema and other pathophysiological responses to the injury. In addition, as any surgeon knows, the adult periosteum is often a flimsy structure and, therefore, damage to it may not be obvious in many patients.

Fortunately, examination of periosteal damage may be carried out in experimental animals where the periosteum is a thick and distinct structure (Ham & Harris 1971). Although this requires dissection to expose the fracture site which may cause additional damage, an adequate impression of soft tissue damage may be provided. With experience, the long bones of experimental animals may be subjected to a similar variety of forces observed in clinical practice as shown in section 3.1 and also described by Alms (1961).

In addition, injection techniques, perhaps the only methods available for anatomical study of vascular damage, can be carried out in experimental animals. The techniques, particularly using the radiopaque medium barium sulphate, have been extensively applied to bone research by many workers notably Gothman (1961), Rhinelander (1968), Trueta (1968) and Brookes (1971). Vascular perfusion is relatively uncomplicated, reproducible and suitable for regular use (Barclay 1951, Sevitt 1981). Nevertheless, perfusion may
further damage already traumatised vessels so that only a proportion of the vessels present are visualised.

In this study both visual and perfusion techniques have been used to examine periosteal, marrow and vascular damage immediately after fracture in a spectrum of tibial shaft fractures. These fractures were produced manually in dead animals.

3.22 Methods

A spectrum of tibial shaft fractures was produced manually in 20 freshly killed mature (Masoud et al. 1986a & b) male New Zealand white rabbits. Each animal was more than 20 weeks old and weighed between 3.5 and 4.5kg. All animals were killed by pentobarbitone overdose and used 1 to 2 hours after death. The medial side of both lower limbs and the anterior abdominal wall were shaved.

A padded clamp was applied to each end of the leg as shown previously in Fig. 36 and the intact hindlimb on the right side was subjected to an angulatory force until the bone fractured. The left intact hind limb was subjected to a spiral force. No attempt was made to measure the fracture force in these experiments since in the clinical situation, fractures of the tibial shaft are not produced by standardised forces. Radiographs of the fractured tibiae were obtained using a standard medical X-ray machine (60 kV, 55mA, 80cm FSD, 0.4 sec. exposure time) with Trimax screened double emulsion medical x-ray films which were hand processed.
The animals were divided into two groups of 10 animals providing 20 fractures each. In the first group, the tibia was fractured and the fracture site was inspected and photographed by exposing the subcutaneous border via a longitudinal anteromedial incision. The tibialis anticus muscle was retracted backwards out of the way but if necessary it was detached distally at the level of the ankle joint and this allowed retraction without tension. This was a descriptive study and, therefore, no attempt was made to measure the dimensions of periosteal laceration or the angles they subtend to the longitudinal axis of the tibia. No attempt was made to observe the whole circumference of the shaft as this would have severed all musculo-periosteal vascular connections.

Next, the abdominal aorta and inferior vena cava were exposed and cannulated via an anterior trans-abdominal approach. 100 to 200ml of 40% aqueous barium sulphate suspension was perfused into the aorta via a standard intravenous giving set with the reservoir kept 1 metre above the operating table. The inferior vena cava was drained simultaneously. These precautions were to ensure perfusion at a physiological pressure which was measured at between 50 and 70mm of mercury at a preliminary study using pressure transducers. A radiograph of the hindlimbs was obtained to certify that the main arteries to the limbs had been perfused (Fig. 41).

The tibiae were excised, fixed in 10% buffered formalin for three weeks and decalcified in formic acid for another
week. After decalcification, the fracture fragments were re-aligned and radiographed together in order to show the relationships in situ. Transverse sections, 0.5 to 1mm thick, of both proximal and distal fracture fragments were cut by hand with a scalpel and radiographed in the Hewlett Packard Faxitron 43855A cabinet X-ray system (at 25kV, 5mA, 68.4cm FSD, 1 minute exposure time). Non screened single emulsion mammography films (CRT 7, 3M) were used and processed by automation. The micro-radiographs so obtained were examined and photographed using the Olympus stereoscopic microscope SZ-Tr.

In the second group, a 10cm long 18G spinal needle was introduced into the medullary cavity percutaneously through the knee joint. The tibia was radiographed to ensure that the needle was correctly inserted. No attempt was made to place the needle at exactly the same location in each tibia since a preliminary study suggested that the marrow was one continuous cavity. The bone was fractured and radiographed. 5 to 10ml of 40% aqueous barium sulphate suspension was injected slowly by hand into the marrow cavity and plain radiographs of the perfused bone were obtained. The fracture site was then inspected as above.

3.23 Results

Plain radiographs of the fractured tibiae before injections revealed that manually applied forces produced a spectrum of fractures as shown previously in Fig. 38. Numerically, angulatory forces produced 6 simple transverse
fractures, 7 comminuted transverse fractures and 7 oblique fractures. Spiral forces produced spiral fractures in all cases. 14 of the 20 spiral fractures were comminuted.

Table 39 shows that angulatory forces produced predominantly transverse or circumferential lacerations of the periosteum (Fig. 42) and Table 40 shows that these fractures were always associated with complete transection of the longitudinally running marrow structures. By contrast, periosteal laceration was longitudinal following spiral fractures (Fig. 43) and the marrow structures often remained in continuity.

Post-decalcification arteriograms of the fractured tibiae (Fig. 44) revealed the presence of dye in all proximal and distal fracture fragments irrespective of the type of fracture (Table 41). Although, from visual inspection, the dye could not have crossed the site of transverse fractures, longitudinally running afferent vessels were perfused with dye in both distal and proximal fracture fragments. Transverse sections confirmed the presence of dye in both distal and proximal stumps of the divided nutrient artery even in transverse fractures (Figs. 45 & 46).

Table 42 shows the results of osteomedullography, which may simulate bleeding from the marrow cavity. The technique revealed more extensive leakage of perfused dye at the site of transverse/oblique fractures (Fig. 47). Whereas, the leakage of dye was usually more restricted in spiral fractures (Fig. 48).
3.24 Discussion and conclusions

These results confirm a close association between fracture morphology and mechanism of injury which has been demonstrated by several workers (Alms 1961). More importantly, however, the results also show a close relationship between fracture morphology and the pattern of damage sustained by the periosteum and the marrow structures. If either or both of these determine the method by which fractures heal (Gallie & Robertson 1928, Potts 1933, Ham 1930, McKibbin 1978), then the method by which a particular fracture heals depends upon its morphology. For instance, healing could be by the periosteal route alone where the periosteal envelope is relatively intact as in spiral fractures. On the other hand, in transverse fractures where the fracture site may be denuded of periosteal cover, healing could involve mesenchymal cells from the adjoining soft tissues through the phenomenon of osteoinduction (Urist 1965). However, it is not proven from the results of this experiment that the speed of healing is affected by the type of periosteal/marrow damage.

Fracture and injection were carried out after the death of animals and, therefore, physiological adjustments to the osseous circulation were prevented. Nonetheless, the branches of the nutrient artery were perfused even though the artery had been transected at the site of the transverse fracture (Figs. 49, 50 & 51). This implies that an open communication exists between the nutrient artery and the periosteal and epiphyseo-metaphyseal arteries (Ficat & Arlet 1980). Brookes
(1971) believes that this communication is formed by intracortical vessels acting as an intermediate capillary bed between the osseous circulations. Most authors believe that through these anastomoses periosteal circulation may compensate for deficient nutrient artery circulation (Rhinelander 1974, Trueta 1968, Brookes 1971). Consequently, diaphyseal nutrition may not be affected by fracture even when the main nutrient arterial trunks have been transected, and if so, it is difficult to implicate inadequate blood supply in the aetiology of delayed or non union as is frequently inferred in clinical practice. However, this conclusion is valid only if the anatomical demonstration of blood vessels, particularly in post mortem materials, is synonymous with blood supply. Therefore, the results of these experiments need to be confirmed by other methods which directly measure blood flow.

Although the amount of bleeding after fracture varies considerably, osteomedullography, which may simulate bleeding from the marrow cavity, suggests that bleeding from this route may be more severe in transverse compared to spiral fractures. Consequently, patients with transverse fractures may be more susceptible to developing acute hypovolaemia and traumatic shock and the relative sizes of the fracture haematoma may be larger than those in spiral fractures. Thus, whether one believes that the fracture haematoma aids repair (Gallie & Robertson 1920, Potts 1933) or hinders it (Ham 1930, Sevitt 1981), its influence on the healing of transverse fractures may be greater than that on the healing of spiral fractures.
The finding of incomplete marrow damage in some fractures also invalidates the use of osteomedullography in the assessment of fracture healing. In this investigation it is assumed that all fractures transect the longitudinally running medullary vessels and thus, interrupt the longitudinal flow of blood within the marrow (Gupta et al. 1980). Therefore, if an injected dye was found to cross the line of fracture, re-vascularisation was assumed to have occurred. This study shows that the medullary structures are grossly intact after spiral fractures and, therefore, the previous assumption cannot be made.

This study may be criticised on four counts. First, the fracture force varied from fracture to fracture. However, manual fracture was deliberately chosen for this study in an attempt to simulate what happens in clinical fractures. In clinical practice, the forces causing tibial shaft fractures are variable and uncontrolled. Second, the damage to the periosteum was not completely visualised in the attempt to preserve musculo-periosteal vascular connections. This is not important because this was a qualitative study. The object was to observe the types of damage sustained after different types of fractures. Third, further significant damage may have been sustained to the soft tissues during the radiographic procedures or during the dissection for inspection or excision. Similar problems arise in clinical practice as the patient is handled at the scene of accident or in the hospital. Fourth, dissection prior to perfusion may have reduced the number of perfusable vessels. This appeared not to have affected the results significantly since the main trunks
of the nutrient artery were filled in all cases. In spite of these possible shortcomings, this study contributes information to the current debate concerning delayed union of fractures. Specifically, it shows that transverse and spiral fractures are associated with their own distinctive patterns of soft tissue damage.
Table 39: Frequency distribution of periosteal laceration in manually produced fractures.

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>Longitudinal laceration</th>
<th>Transverse laceration</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple transverse</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Angulatory force</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oblique transverse</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Comminuted transverse</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Spiral force</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple spiral</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Comminuted spiral</td>
<td>2</td>
<td>6</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>7</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 40: Frequency distribution of marrow damage in manually produced fractures.

<table>
<thead>
<tr>
<th></th>
<th>Incomplete damage</th>
<th>Complete damage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple transverse</td>
<td>-</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Angulatory force</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oblique transverse</td>
<td>-</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Comminuted transverse</td>
<td>-</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Spiral force</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple spiral</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Comminuted spiral</td>
<td>8</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>
Table 41: Frequency distribution of microarteriographic findings in manually produced fractures.

<table>
<thead>
<tr>
<th>Intramedullary vessel filling</th>
<th>Proximal</th>
<th>Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple transverse</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Angulatory force Oblique</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Comminuted transverse</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Spiral force Simple spiral</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Comminuted spiral</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 42: Frequency distribution of osteomedullographic findings in manually produced fractures.

<table>
<thead>
<tr>
<th>Extravasation</th>
<th>Minor</th>
<th>Moderate</th>
<th>Gross</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple transverse</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Angulatory force Oblique</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Comminuted transverse</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Spiral force Simple spiral</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Comminuted spiral</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>20</td>
</tr>
</tbody>
</table>
Figure 41  Arteriogram of a rabbit hindlimb with a comminuted transverse fracture of the tibia (arrowed) created manually after death showing that the main limb arteries have been perfused
Figure 42 Photograph showing periosteal laceration by a transverse fracture created manually after death. The arrows point to the edges of a transverse tear in the periosteum, which have retracted a few mm from the fracture site.
Figure 43  Photograph showing periosteal laceration by a spiral fracture created manually after death. The arrows point to the edges of a longitudinal tear in the periosteum.
Figure 44 Arteriogram (post-decalcification) performed immediately after fracture in a dead animal showing branches of the nutrient artery (arrowed) - fracture fragments re-aligned to show relationship in situ

Figure 45 Micro-arteriograph (post-decalcification) of a transverse section through the proximal fragment of a transverse fracture of the rabbit tibia created manually after death
Figure 46 Micro-arteriograph (post-decalcification) of a transverse section through the distal fragment of a transverse fracture of the rabbit tibia created manually after death.

Figure 47 Radiographs of an oblique fracture created after death before (left) and after (right) perfusion of the marrow.
Figure 48 Radiographs of a spiral fracture created after death before (left) and after (right) perfusion of the marrow
3.3 SUMMARY

It is widely believed that the mechanism of healing may depend on fracture morphology. However, it has never been proved whether this is due to the fact that different fracture types sustain different types of soft tissue damage. This study has attempted to answer this question by comparing periosteal, marrow and vascular damage following transverse fractures with that in spiral fractures. These are fractures produced by different mechanisms (Alms 1961) and some authors believe that they heal differently (Wilson 1981, Johner & Wruhs 1983). The results show that transverse fractures may be associated with greater damage to the surrounding soft tissues than spiral fractures. Furthermore, the damage in transverse fractures may also be qualitatively different from that in spiral fractures. Transverse fractures produced circumferential laceration of the periosteum and complete transection of the marrow but spiral fractures produced longitudinal periosteal laceration and incomplete marrow damage. Interestingly, although the nutrient artery may be interrupted by fracture, perfusion of its main trunks on either side of, and up to, the fracture line may still be observed due to filling via available collateral channels.
4.0 EXPERIMENTAL STUDIES OF FRACTURE HEALING
4.1 AN INVESTIGATION OF THE HEALING OF AN EXPERIMENTAL FRACTURE OF THE MATURE RABBIT TIBIAL DIAPHYSIS IN THE ABSENCE OF MEDULLARY TISSUES.

4.11 Introduction

Accounts of fracture repair usually describe the new bone formed at the fracture site as being derived from three potential sources; namely, extraskeletal (including periosteal), endosteal and intracortical sources (McKibbin 1978). The relative contribution of each source to the repair does not appear to have been studied previously in a satisfactory manner. This can be investigated by excluding each osseous tissue in turn completely from the site of a fracture (Fig. 49a) and observing healing after the exclusion procedure. Reaming and nailing should destroy the endosteal source of new bone and extraskeletal application of a polythene sheath should prevent the periosteum from participating in the repair of a fracture. By these different methods, fracture healing in the absence of each of these tissues may be studied at various time intervals after the tissue exclusion procedure (Fig. 49b).

It is obvious that extra-medullary osteogenesis and intracortical osteogenesis are the only pathways available for union of fractures after the destruction of the marrow (Yu 1960, Anderson 1965, Richany et al. 1965) as occurs after intramedullary nailing. However, there is no clear consensus on the biological processes involved perhaps because most studies are primarily concerned with the vascular changes. Some,
notably Trueta and Cavadas (1955) and Kunstcher (1968), believe that intramedullary nailing stimulates bone formation but others, notably Anderson (1965), propose that it reduces the healing potential of bone. On the other hand, Fitts et al. (1949) postulate that the destruction of the marrow alone has no influence on bone formation.

Sevitt (1981) claims that union of nailed fractures is preceded by fibrous bridging of bony collars around the proximal and distal fragments and by extension of fibrosis between the fractured cortices. This is eventually converted to bone as the bony collars fuse. Hence, nailed fractures heal by multiple semi-confluent or isolated foci of new bone. By contrast, Anderson and his colleagues (1962, 1965) claim that union is primarily by endochondral ossification externally, although direct formation of bone can occur with tight fitting nails. Danckwardt-Lilliestrom (1969) believes that two kinds of new bone are formed after the destruction of the marrow: thin layers of lamellar bone laid down directly on to the periosteal surface of the cortex and immature (woven) bone elsewhere. He claims that the lamellar bone presents as either circumferential lamellae or new primary osteons and regards it as resumed or accelerated normal bone formation. He further claims that the woven bone, which is apparently more prominent, is related to subperiosteal haemorrhage which he associates with reaming. By contrast, Henricson et al. (1987), who also believe that two types of callus are formed after nailing, suggest that the first callus is due to proliferation by predetermined cells and the second to cartilage conversion of cells migrating in from outside.
The present study attempts to resolve these controversies by examining the successive biological stages in fracture healing in the absence of medullary tissues. The aim was to determine whether the absence of the medullary tissues affects fracture healing.

4.12 Methods

Three mature male New Zealand white rabbits were killed by pentobarbitone overdose. A transverse osteotomy was created in each tibia and, in addition, the medullary cavity of the right tibia was reamed and nailed in order to completely eliminate the medullary tissues. The blood supply to the fragments was investigated by arteriography to provide baseline data for the recovery experiments.

In a parallel study, under general anaesthesia and using aseptic techniques, a transverse osteotomy was created in the right tibia of each of 12 live mature male New Zealand rabbits. The marrow cavity was reamed and an intramedullary nail was passed retrogradely. The animals were allowed to recover and were housed in standard pens and fed a standard diet. Groups of 3 animals were investigated one day, one week, two weeks and four weeks respectively after operation. Two animals were investigated by arteriography by perfusing the aorta under general anaesthesia. In one animal, the operated tibia was investigated by osteomedullography after death by pentobarbitone overdose. Fracture healing was examined histologically in all cases.
Choice of experimental animals:

The rabbit was chosen as the experimental animal because it is frequently used for experiments on fracture healing and, therefore, a lot of data is available with which to compare results. Furthermore, the tibia is large enough to allow various manipulations to be carried out and the animal is relatively cheap so that a reasonable number could be used. Locally, many investigators use rabbits for their research and, consequently, 50 rabbits were available to the author for practice runs.

Only male animals were used to reduce the influence of constitutional factors on the results. Mature animals were used since in the clinical studies only adult tibial shaft fractures were investigated. Maturity in the rabbit was defined according to the criteria of Masoud et al. (1986a & b) and when the iliac apophysis was closed and the animals weighed between 3.5 and 4.5kg.

Male New Zealand white rabbits were bought from various breeding centres and acclimatised at the Biomedical Services Unit, University of Leicester, over a period of at least 3 weeks. The animals were caged individually and were fed a standard diet containing calories, proteins, minerals and vitamins. The animals were also given water *ad lib*. The lighting cycle was 12-hours-off-12-hours-on.

In this study, 1 day, 1 week, 2 weeks and 4 weeks
respectively were selected as the observation intervals. Post mortem studies were also carried out to provide baseline data for the recovery experiments.

Anaesthesia:

Anaesthesia was accomplished by premedication with 10mg midazolam ("Hypnovel", Roche) given into the marginal ear vein followed by induction of anaesthesia with 5% Halothane and nitrous oxide and oxygen (2:1). Anaesthesia during surgery was maintained with a nitrous oxide, oxygen and 2 - 3% halothane mixture by face mask. Respiration and heart rates were monitored continuously during surgery.

Operative procedures:

Standard aseptic surgical techniques were employed in all survival experiments and the right tibia only was operated on in all cases. The operation site was shaved and isolated using plastic bags. The skin was sterilised with povidone iodine solution ("Betadine surgical scrub", NAPP Laboratories) and with chlorhexidine gluconate ("Hibitane", ICI).

For intramedullary nailing, the subcutaneous border of the middle third of the tibia was exposed through an anteromedial incision. The skin edges and the muscles were retracted away from the shaft at the proposed osteotomy site by inserting McDonald's dissectors. The periosteum was not incised or stripped prior to a transverse osteotomy which was created in the lower half of the tibia using a fine-toothed,
air-powered reciprocating/sagittal saw (Zimmer Micro 100). The saw blade was 100u thick to reduce thermal burns to the bone and bone cutting was carried out under continuous normal saline irrigation. The medullary cavity was reamed proximally and distally from the osteotomy site. An opening for the insertion of the nail was obtained by allowing the reamer to extrude proximally through a second wound over the anterior tibia. The nail (a 10cm long, 1mm thick Kirschner wire or 2mm thick Steinmann’s pin) was introduced retrogradely, the osteotomy was reduced and the nail was then fully introduced into the medullary cavity.

Duplocillin 0.25ml (75mg procaine penicillin and 56.25mg benzathine penicillin, Gist-Brocades) was infiltrated into the wound locally before closure. The wound was closed in layers with interrupted 4/0 coated vicryl ("polyglactin 910", Ethicon) to the deep fascia and continuous 3/0 supramid (white, pseudo-monofilament polyamide", BBM-AG) to the skin.

Analgesia:

Analgesia was routinely given using buprenorphine ("Temgesic", Reckitt & Colman) 0.1mg 12 hourly intramuscularly for the first three days. The animals were closely monitored for adverse effects post-operatively based on the methods of assessment described by Morton and Griffiths (1985).

Perfusion techniques:

This was an anatomical study and, therefore, the part
played by vessels in fracture healing was investigated by vascular perfusion techniques; namely, arteriography and also osteomedullography, which was used to reveal when the marrow was reconstituted after fracture. Barium sulphate suspension was used because it has been shown by several workers (Gothman 1961, Rhinelander 1972) to be a particularly suitable injection medium for this type of study. It is capable of demonstrating vessels of various sizes down to fine calibre vessels with excellent resolution.

The technique of arteriography was similar to that described by Barclay (1951) and by Sevitt (1981). In the recovery experiments, the animal was given 1000 units of heparin with the premedication. Under general anaesthesia, the abdominal aorta and the inferior vena cava were cannulated using an 18G plastic abrocath needle via an anterior abdominal approach. The aortic cannula was connected to a gravity feed system comprising of a standard intravenous giving set and a 200ml reservoir (see section 3.2). The vena cava was connected to an outlet tube. The reservoir was filled with 40% aqueous barium sulphate suspension (Baritop 100 suspension 0.5 – 0.1v finely dispersed particles, Concept Pharmaceuticals, Amersham). The inferior vena cava was drained simultaneously as the aorta was being perfused. These precautions ensured arterial perfusion at a physiological pressure which was measured at between 50 and 70mm of mercury as indicated previously in section 3.2. Towards the end of perfusion, the infusion rate decreased gradually and the animals often died spontaneously. Muscle spasms were observed in the lower limbs in all animals shortly after the commencement of perfusion. Although dye was
usually observed in the fine vessels on the surface of muscles and in the skin, the limbs were radiographed after perfusion to confirm that the main limb arteries had been penetrated.

The technique of osteomedullography was similar to that described by Thomas et al. (1982) but it was carried out after the death of the animal in all cases. The animal was given 1000 units of heparin into a marginal ear vein and a few minutes later a lethal dose of pentobarbitone was given. A 10cm long 18G spinal needle was introduced into the medullary cavity, by the side of the intramedullary nail, percutaneously through the knee joint. The needle was advanced 1 to 2cm beyond the cancellous bone of the proximal metaphysis and up to 10ml of perfusate was injected slowly by hand into it. The injection pressure was not measured as the intention was to demonstrate all available efferent channels. A radiograph of the perfused bone was obtained after the injection.

Preparation of specimens:

Perfused bones were excised together with a cuff of adherent soft tissues. The nails were removed and the bones were fixed in 10% buffered formalin for 3 weeks. Next, the bones were decalcified in formic acid for 7 to 10 days.

Only a 5cm length of the tibia with the osteotomy site at its centre was examined by histology and by micro-radiography. The specimens were sectioned according to the scheme in Fig. 50 so that the radiographic image could be correlated with the histology. The 0.5 to 1mm thick sections for radiography and
the thicker blocks for histology were cut by hand using an ordinary surgical scalpel. The tissue blocks were processed automatically in the Shandon 2LE tissue processor using a 48 hour cycle with 4 hourly emersion in graded alcohol from 70% to absolute followed by 3 four hourly changes of chloroform, then 4 hours in fibrowax and finally another 4 hours under vacuum in fibrowax. Next, the specimens were blocked out and embedded in fibrowax. The embedded specimens were cut on the Anglia Scientific base sledge microtome Type 200 using a super tough wedge-shaped knife at 7μ thickness. The slides were heated at 60°C overnight in an incubator then stained automatically in the Shandon Linistain GLX using Mayer's haematoxylin and eosin stains.

**Radiography:**

Plain radiographs of limbs were obtained post-operatively and before and after perfusion in the theatre using a standard medical x-ray machine (60kV, 55mA, 80cm film-source-distance or FSD, 0.4 sec. exposure time). Trimax medical x-ray films were used and these were hand-processed at room temperature.

Radiographs of decalcified specimens and thin sections of decalcified specimens were obtained in the Hewlett Packard Faxitron 43855A cabinet or desk top system (20 - 25 kV, 5mA, 68.4cm FSD, 1 min. exposure time) using non screened single emulsion mammography films (CRT 7, 3M). The films were processed automatically and the radiographs were examined and photographed in the Olympus SZ-Tr stereomicroscope.
4.13 Results

All of the animals survived for the planned duration of experiments and they bore weight on the operated leg within hours after operation. The operation wounds healed within a few days and there were no wound infections. A number of osteotomies initially showed some rotational movement but this ceased within a few days.

Radiographic examination immediately post-operatively revealed good alignment of the fracture fragments in all cases (Fig. 51). Radiographic examination of day-old and week-old osteotomies at the time of death revealed no callus formation. At two and at four weeks, a thin layer of radiological external callus was observed in all cases at the osteotomy site and the fractures appeared to be united.

Visual inspection of day-old osteotomies at the time of death revealed extensive bleeding had occurred into the soft tissues far beyond the vicinity of the osteotomy. At one week, the tissues were extensively discoloured and there was extensive interstitial oedema and tissue swelling. At two and at four weeks, the haematoma, oedema and swelling had subsided and the soft tissues were adherent to the osteotomy sites. A diffuse thickening was often palpable at the osteotomy sites extending 1 or 2cm proximally and distally.

It was often difficult to re-align the two fragments of a specimen anatomically or to obtain more than one longitudinal section for micro-radiography, in specimens where the osteotomy
had not healed (i.e. at Day 1 and at Week 1), after the nail had been removed in order to process the specimen.

Post-decalcification arteriograms of tibiae which had been osteotomised after death but not nailed revealed dye penetrating the main trunks of the nutrient artery on either side of the osteotomy line (Fig. 52a). Most of these medullary vessels were no longer observed after reaming and nailing (Fig. 52b).

Post-decalcification micro-arteriographs of specimens obtained from the recovery experiments (Fig. 52c to 52e) revealed the successive changes in the vascularisation at the osteotomy site. One day post-operatively, there was significant vascular perfusion only in the proximal fragment (Fig. 52c). At 1 and 2 weeks, a profuse number of periosteal vessels could be observed covering the surface of the fractured bone (Fig. 52d). These vessels appeared to be better perfused at 4 weeks (Fig. 52e) and some perfused vessels could be observed to penetrate the cortex centripetally and others appeared in the medullary cavity further away from the osteotomy line.

Micro-radiography after injecting dye into the marrow cavity revealed that the perfused dye penetrated the whole length of the marrow in all cases through the nail track. No venous channels were filled either in the marrow or externally. Therefore, osteomedullography was not found to provide any useful information.

The histological sections from the recovery experiments
(Fig. 53) revealed successive stages in the formation of the external callus. In relevant post mortem materials and at 1 day, most of the marrow was shown to have been removed in all cases (Fig. 53a). One week post-operatively, in all cases, a thin and continuous layer of new (woven) bone of uniform thickness had formed in the cambium layer of the periosteum next to the shaft (Fig. 53b). Its trabeculae were orientated perpendicularly to the shaft. The periosteum was several layers thick and its cells could be observed to be continuous with the new bone and with cartilaginous masses near to, and on either side of, the osteotomy site. There was no distinct boundary between subperiosteal new bone and the cartilaginous masses. The cartilage cells appeared to increase progressively in size the closer they were situated to the cortex where some had become degenerate. The arrangements in the cartilaginous masses appeared to be similar to that usually observed in growth plates (Fig. 53c). A sub-periosteal haematoma was not observed in any specimen on day 1 or week 1.

The proliferating periosteum could be clearly distinguished from the surrounding fibrous connective tissue in its darker staining, increased cellularity, mitotic bodies, smaller cells and finer fibrous elements. In some sections, periosteal reaction and proliferation appeared to be absent from the first few millimetres immediately bordering the osteotomy line as noted by Henricson et al. (1987).

Two weeks post-operatively, subperiosteal new bone and cartilage formation was observed as above in all cases (Fig. 53d). In addition, the cartilaginous masses were more mature
and vascular channels surrounded by areas of endochondral ossification could be observed at several foci within the cartilage (Fig. 53e). The periosteum had become reconstituted over the osteotomy site and beneath this could be observed fibrous/granulation tissue which had bridged the cartilaginous masses on either side of the fracture. At the periphery of this fibrous/granulation tissue some of the cells were observed to be continuous with adjacent areas of new cartilage formation. These foci, presumably representing cartilage transformation, were often highly vascularised (Fig. 53f). Furthermore, the cartilaginous masses did not appear in any of the sections to fuse directly as suggested by Ham (1930).

At four weeks, the osteotomy gap had been bridged externally with bone, cartilage or with both. A V-shaped cartilaginous mass, with its apex at the fracture cleft and its base underneath the repaired periosteum, could be observed in some sections (Fig. 53g). In addition, foci of osteoclastic activity (remodelling) could be observed within the new bone. Union in one of the three tibiae examined at this time interval did not appear to have involved cartilage formation at all (Fig. 53h).

Endosteal callus was observed in some sections (see Figs. 53a to 61h) within the spaces between the nail and the endosteal surface of the cortex in either distal or proximal fragments or in both but the fracture gap, the space directly between the bone ends, was not filled in all specimens.

4.14 Discussion and conclusions
This study has attempted to show the healing of a transverse osteotomy following exclusion of marrow tissues. Healing occurred between 2 and 4 weeks after operation by periosteal callus formation. Other workers have shown that this is the time scale for healing in the rabbit tibia following closed treatment without specific tissue exclusion (Koekenberg 1963). It may, therefore, be concluded that the medullary tissues are not critical to healing since delayed union is not caused by their absence.

The post mortem perfusion studies confirmed the findings in section 3.2, where fractures were manually created, that a fracture may not directly deprive fracture fragments of a blood supply because of available collateral channels. These studies also showed that reaming and nailing cause extensive damage to the blood supply. In recovery experiments, afferent vessels in the nailed distal fragment were not perfused with dye thus suggesting additional damage perhaps due to post-operative oedema. Whereas from 1 week onward, both fragments were well vascularised via the periosteum.

The histological studies demonstrate certain histogenetic aspects of fracture healing in the absence of the marrow tissues. The early stages consist of intense periosteal proliferative activity resulting within days in the formation of a thin layer of bone "cemented" to the old cortex. This is presumably the primary callus response discussed by McKibbin (1978) and referred to as subperiosteal bone collar by Koekenberg (1963). Both Dankwardt-Lilliestrom (1969) and
Henricson et al. (1987) also appear to have recognised it but the new bone formation was not observed to be due to subperiosteal haemorrhage as claimed by the former author. Later, in addition to subperiosteal new bone formation, endochondral ossification is observed close to the fracture site and the osteotomy gap is bridged externally. This has been regarded as the secondary callus response by several workers (Dankwardt-Lilliestrom 1969, McKibbin 1978, Henricson et al. 1987).

In this study, the various histological features described above (subperiosteal new bone formation, endochondral ossification and repair of periosteum) appeared to be a continuum rather than multiple stages of healing as proposed by some workers, notably McKibbin (1978) and Henricson et al. (1987). Furthest from the fracture site, there is subperiosteal new bone which merges, nearer to the fracture site, with proliferating cartilage. Thereafter, the cartilage undergoes enchondral ossification. At the fracture site itself, the reaction is related to the repair of the periosteum with granulation tissue. After the periosteum has been reconstituted, the repair tissue undergoes bone and/or cartilage conversion. Because there were three cell types related to the proliferating periosteal cells, it may be concluded that they gave rise to the three different cell types; namely, fibroblastic to repair periosteal tear at the fracture gap, osteoblastic and chondroblastic but the reasons for three divergent differentiation pathways is not clear. Histologically, cartilage formation did not appear to be related to a poor blood supply as claimed by Ham (1930). In
fact, the highly vascular granulation tissue is ultimately converted to avascular cartilage which then undergoes re-vascularisation during endochondral ossification.
Figure 19a. Diagram showing the experimental model for investigating healing of fracture of the diaphyseal cortex.

- Transverse diaphyseal osteotomy
- Nailed (periosteal healing)
- Ensheathed (endosteal healing)
- Nailed and ensheathed (intracortical healing)
Figure 49b Flow diagram of experimental procedures for investigating fracture healing
Figure 50  Diagram of schema for sections

3 x 1 mm A  B
thick slices for histology sections
for X-rays
Figure 51
Radiograph of an osteotomised and nailed rabbit tibia showing site of osteotomy (arrowed) and intramedullary nail.

Figure 52a
Arteriogram (post-decalcification) performed immediately after osteotomy in a dead animal, showing branches of the nutrient artery (arrowed). The fragments have been re-aligned to show their relationship in situ.
Figure 52b
Micro-arteriograph (post-decalcification) of a longitudinal section through a reamed and nailed osteotomy created after death, showing perfusion of marrow and some cortical afferent vessels in both fragments (the proximal is marked with a pin)

Figure 52c
Micro-arteriograph (post-decalcification) of a longitudinal section through a reamed and nailed osteotomy created in a live animal 1 day after operation, showing significant perfusion only in the proximal fragment (marked with a pin)
Figure 52d.
Micro-arteriograph (post-decalcification) of a longitudinal section through a reamed and nailed osteotomy created in a live animal 1 week after operation, showing numerous perfused vessels on the external surfaces of the fragments.

Figure 52e
Micro-arteriograph (post-decalcification) of a longitudinal section through a reamed and nailed osteotomy created in a live animal 4 weeks after operation, showing perfusion of periosteal, cortical and medullary vessels.
Figure 53a Photo-micrograph (H & E) of a longitudinal section through a reamed and nailed osteotomy created in a live animal 1 day after operation, showing that most of the marrow has been removed (mag x 5)

Figure 53b Photo-micrograph (H & E) of a longitudinal section through a reamed and nailed osteotomy created in a live animal 1 week after operation, showing subperiosteal new bone formation (B) and cartilage formation (C) (mag x 5)
Figure 53c  Photo-micrograph (H & E) of a higher magnification of the area of cartilage formation seen in Fig. 53b showing columnar arrangement of hypertrophic cartilage cells (mag x 100)

Figure 53d  Photo-micrograph (H & E) of a longitudinal section through a reamed and nailed osteotomy created in a live animal 2 weeks after operation, showing subperiosteal new bone (B), cartilage formation (C) and bridging of the fracture gap by fibrous/granulation tissues (F) (mag x 5)
Figure 53e Photo-micrograph (H & E) of a higher magnification of the area of endochondral ossification seen in fig 53d, showing foci of bone formation in relation to blood vessels (brown staining) within the cartilage masses (mag x 160).

Figure 53f Photo-micrograph (H & E) of a higher magnification of the area of repaired periosteum over the fracture site seen in Fig 53d, showing foci of cartilage formation from highly vascularised fibrous/granulation tissues (mag x 160). The blood vessels are stained brown because they contain barium sulphate.
Figure 53g Photo-micrograph (H & E) of a longitudinal section through a reamed and nailed osteotomy created in a live animal 4 weeks after operation, showing the osteotomy gap bridged externally by bone and cartilage (mag x 5)

Figure 53h Photo-micrograph (H & E) of a longitudinal section through a reamed and nailed osteotomy created in a live animal 4 weeks after operation, showing the osteotomy gap bridged externally by bone alone (mag x 5)
4.2 AN INVESTIGATION OF THE HEALING OF AN EXPERIMENTAL FRACTURE OF THE MATURE RABBIT TIBIAL DIAPHYSIS IN THE ABSENCE OF PERIOSTEAL TISSUES.

4.21 Introduction

There is little doubt that certain cells in the bone marrow can form bone. Healing at sites such as the neck of femur or the patella where the periosteum is virtually absent, may depend entirely on the marrow cells. Fractures through the red marrow of the vertebrae heal faster than fractures through areas of yellow fatty marrow in the shaft. Autografts of red marrow have been shown to produce bone when transplanted to various extra-skeletal sites (Urist & McLean 1952, Burwell 1964). The osteogenic potential of banked bone has been increased by impregnating it with autologous marrow (Burwell 1966). These cells are probably responsible for the new bone formation that is commonly observed in the marrow during diaphyseal fracture healing.

However, the importance of medullary osteogenesis to diaphyseal fracture healing is not clear. Some authors believe that it is critical to normal union and Sevitt (1981) has suggested that delayed union is caused by failure of medullary new bone formation to bridge the fracture gap. By contrast, Cavadias and Trueta (1965) concluded from their series of experiments using vascular isolation techniques, that endosteal callus is not important for the normal union of fractures. However, these latter authors excluded many of their animals in
which the operative manoeuvre resulted in ischaemia of bone. No histological or micro-angiographic results were presented for these animals and only two adult bones were reported in the entire series. Therefore, a gap still exists in our knowledge of healing in the absence of periosteal tissues particularly of adult bones.

In the present study, a similar technique to that of Cavadas and Trueta (1965) has been used to examine fracture healing in the absence of the periosteal tissues. The aim was to determine whether the absence of periosteal tissue affects fracture healing.

4.22 Methods

Three mature male New Zealand white rabbits were killed with pentobarbitone overdose, a transverse osteotomy was created in the right tibia and a silastic sheath was wrapped around the osteotomy as follows:

The subcutaneous border of the middle third of the tibia was exposed through an anteromedial incision. The skin edges and the muscles were retracted away from the shaft at the proposed osteotomy site and the periosteum was stripped for 1 - 2 cm on either side of this. A 3 cm long semi-rigid silastic tube was cut open length-wise and placed around the shaft. Two McDonald's dissectors were introduced between the sheath and the shaft and a transverse osteotomy was created in the shaft using a fine-toothed, air-powered reciprocating/saw sagittal saw (Zimmer Micro 100). The saw blade was 100u thick to reduce
thermal burns to the bone and bone cutting was carried out under continuous normal saline irrigation. The dissectors were removed and the sheath was fastened on to the shaft with two cerclage wires; one on either side of the osteotomy. A third wire was sometimes required to prevent ingrowth of vessels into the osteotomy site through gaps between the edges of sheath.

The blood supply was investigated by arteriography (see section 4.1) to provide baseline data for the recovery experiments.

In a parallel study, under general anaesthesia and using aseptic techniques, a transverse osteotomy was created and sheathed in the right tibia of each of 12 live mature male New Zealand rabbits as above. The wound was closed in layers after infiltrating antibiotics locally and a groin to toe plaster of Paris cast was applied to provide extra support. The animals were allowed to recover and were housed in standard pens and fed a standard diet. Groups of 3 animals were investigated one day, one week, two weeks and four weeks respectively after operation. Two animals were investigated by arteriography and the perfusion of the aorta was carried out under general anaesthesia (see section 4.1). In one animal the operated tibia was investigated by osteomedullography after death by pentobarbitone overdose (see section 4.1). Fracture healing was examined histologically in all cases (see section 4.1), sectioning being performed as in Fig. 51.

4.23 Results
All animals survived without distress for the planned duration of experiment and none developed wound infection. The plaster casts were well tolerated.

Immediate post-operative radiographic examination (Fig. 54) revealed a minor degree of angulation in some cases. No callus formation was observed at the osteotomy site in any of the animals.

Extensive haemorrhage and inflammatory reactions were observed visually in the operated legs in the animals killed at the earlier intervals. The silastic sheaths excited a vigorous local fibrous tissue reaction and the proximal and distal ends of the sheath were usually enclosed in a layer of new bone at the later intervals.

Post-decalcification micro-arteriographs of specimens obtained following osteotomy and application of a sheath in dead animals showed that the main trunks of the nutrient artery, up to the osteotomy line in both fragments, and some of their transverse branches had been perfused in all cases (Fig. 55a). Some of these vessels were still perfused at Day 1 and at Week 1 after operation in the recovery experiments but vascularisation appeared reduced in the distal fragments (Fig. 55b). At 2 and at 4 weeks, vessels were perfused in the proximal but not in the distal fragments (Fig. 55c).

Micro-radiography after injection of dye into the marrow cavity at Day 1 and Week 1 revealed that the dye had penetrated the marrow up to the fracture line where there was some
pooling. No significant numbers of cortical efferent vessels were perfused. After 2 weeks, dye perfused into the marrow no longer reached the osteotomy line and, in addition, no significant numbers of efferent vessels were perfused.

Macroscopic and histological examination of excised specimens revealed non union of the osteotomy in all cases. At day 1, there was haemorrhage at the fracture site (Fig. 56a). No physical damage was observed to have occurred to the marrow adjacent to the osteotomy site in all cases (Fig. 56b). At 1 and at 2 weeks, there was a medullary reaction in the proximal but not in the distal fragments (Fig. 56c). In the proximal fragment, away from the fracture site, there was cellular proliferation in the endosteum which was lined with dark staining plumb osteoblasts. Within the marrow cavity, there was increased vascularity with macrophages ingesting fat released from damaged fatty marrow cells. Close to the fracture site, there was granulation tissue within the full width of the marrow cavity and within this tissue there was woven bone formation (Fig. 56d). By contrast, the distal fragment appeared avascular with fat necrosis in the marrow and no tissue reaction (Fig. 56e).

At 4 weeks, in 2 specimens, the difference in the reaction within the marrow of the two fragments was more marked (Fig. 56f). In the proximal fragment, some fibrosis of the marrow could be observed and close to the fracture gap more mature bone was undergoing remodelling (Fig 56g). The marrow of the distal fragment was necrotic and was occupied by acellular and amorphous materials (Fig. 56h). In one specimen, the marrow
in both fracture fragments contained bone, cartilage and fibrous tissue elements but the fracture gap was not bridged with bone or with any other tissues. A periosteal reaction was not observed in any specimen.

4.24 Discussion and conclusions

Considerable difficulties were encountered in obtaining longitudinal sections for micro-radiography because the osteotomies had not healed. At first the sheaths were left in place during fixing and decalcification in order to preserve the in situ relationships between the fracture fragments. However, the sheath prevented the penetration of the bone underneath by the fixative and the decalcifying agent so that they had become putrid at the time of examination. For this reason, all the specimens were abandoned and the study repeated and the sheaths were removed prior to the specimens being fixed in formalin. On the other hand, when the sheaths were removed immediately after obtaining specimens, it was often impossible to re-align the fragments accurately after decalcification because of the smooth surfaces of the osteotomies. In addition, it was often only possible to obtain one or two sections for radiography, not 3 as intended, because the sheath frequently split as the first sections were being cut. Nevertheless, it was still possible to use these methods to observe the anatomy of the vascular adaptations that occurred after fracture in this model.

The post-mortem study showed that the main trunks and transverse branches of the nutrient artery to both distal and
proximal fragments are preserved after a simple osteotomy. This was also the finding in section 3.2 when the rabbit tibia was fractured manually after death. However, the fact that the marrow in 8 out of 9 distal fragments became necrotic from 1 week after operation suggests that the blood supply to the distal fragment may not normally be sufficient to maintain marrow viability particularly when the periosteum had also sustained damage. In fact, the distal fragment was no longer perfused by dye injected into the aorta two weeks after operation in all cases. Nevertheless, in one case, a vital reaction occurred both sides of the fracture gap perhaps indicating there was sufficient blood supply to the distal fragment in that instance. In the study by Trueta and Cavadias (1965), angiography in the two adult animals in the series also showed that only the proximal stump of the nutrient artery was filled. Thus, an effective blood supply may not be available distally close to the fracture site in the absence of the periosteal circulation. Consequently, medullary callus formation did not occur in the distal fragment in 8 out of 9 cases which survived beyond 1 week.

This study has attempted to show the healing of a transverse osteotomy of the adult rabbit tibia in the absence of the periosteum. Angiographic and histological results were presented for 12 adult animals in the recovery experiment. Healing did not occur in all cases during the period of study. However, the proximal fragment, which remained vascularised in all cases, formed normal medullary callus and certain histological features of medullary osteogenesis were demonstrated by this study. Proliferation of endosteal cells
commence within days of fracture. The early stages consist of intense proliferative activity in the endosteum which subsequently spreads to occupy the whole width of the marrow. The repair tissue close to the fracture site differentiates predominantly along osteoblastic pathways with a small amount of cartilage formation. This small amount of cartilage is presumed to undergo endochondral ossification eventually.

The fragments did not unite in any case and there are three possible reasons for this, namely: avascularity distal to the fracture site, absent periosteum and motion at the fracture site. The histological and perfusion evidence discussed above suggest that the likely reason is avascularity of the distal fragment. However, in one case where there was medullary callus formation on both sides of the osteotomy, there was still no bridging. This brings into question as to whether periosteal damage and motion were also involved.

The osteotomy was not rigidly fixed and, therefore, the repair tissue could have been continuously ruptured by persistent motion at the osteotomy site. The use of an external fixator could have prevented this and future experiment should take this into consideration. Nevertheless, there was no histological evidence of motion which is usually indicated by a split in the tissues and its lining by a pseudo-synovial membrane.
Figure 54
Radiograph of an osteotomised and sheathed rabbit tibia, showing site of osteotomy (arrowed) and silastic sheath held in place with cerclage wires

Figure 55a
Micro-arteriograph (post-decalcification) of a longitudinal section through a sheathed osteotomy created after death, showing extravasation at osteotomy site (arrowed) and longitudinal and transverse marrow afferent vessels on either side of the fracture
Figure 55b
Micro-arteriograph (post-decalcification) of a longitudinal section through a sheathed osteotomy created in a live animal 1 week after operation, showing reduced vascularisation in the distal fragment.

Figure 55c
Micro-arteriograph (post-decalcification) of a longitudinal section through a sheathed osteotomy created in a live animal 4 weeks after operation, showing no significant vascularisation in the distal fragment.
Figure 56a Photo-micrograph (H & E) of a longitudinal section through a sheathed osteotomy created in a live animal 1 day after operation, showing haemorrhage at fracture site and a normal marrow (mag x 5)

Figure 56b Photo-micrograph (H & E) of a higher magnification of the area of normal marrow seen in Figure 56a (mag x 25)
Figure 56c  Photo-micrograph (H & E) of a longitudinal section through a sheathed osteotomy created in a live animal 2 weeks after operation, showing medullary reaction in the proximal but not the distal fragment (mag x 5)

Figure 56d  Photo-micrograph (H & E) of a higher magnification of the area of the marrow seen in the proximal fragment in figure 56c, showing new bone formation (B), macrophages ingesting fat globules (M) (mag x 25)
Figure 56e  Photo-micrograph (H & E) of a higher magnification of the area of marrow seen in the distal fragment in figure 56c, showing loss of cellularity (mag x 25)

Figure 56f  Photo-micrograph (H & E) of a longitudinal section through a sheathed osteotomy created in a live animal 4 weeks after operation, showing a medullary reaction in the proximal but not in the distal fragment (mag x 5)
Figure 56g  Photo-micrograph (H & E) of a higher magnification of the area of marrow seen in the proximal fragment in Figure 56f, showing more mature bone (B) and also cartilage formation (C, (mag x 25)

Figure 56h  Photo-micrograph (H & E) of a higher magnification of the area of marrow seen in the distal fragment in Figure 56f, showing acellular amorphous materials (mag x 25)
Figure 56i  Photo-micrograph (H & E) of a longitudinal section through a sheathed osteotomy created in a live animal 4 weeks after operation, showing a medullary reaction in both fragments (mag x 5)
4.3 AN INVESTIGATION OF THE HEALING OF AN EXPERIMENTAL FRACTURE
OF THE MATURE RABBIT TIBIAL DIAPHYSIS IN THE ABSENCE OF
MEDULLARY AND PERIOSTEAL TISSUES.

4.31 Introduction

In section 4.1, an attempt was made to study fracture healing in the absence of the medullary contribution. In section 4.2, fracture healing was studied in the absence of the periosteal tissues. In this study, both medullary and periosteal tissues have been excluded from the fracture site for completeness of the tissue exclusion study. The aim was to determine whether normal fracture healing can occur in the absence of these tissues.

4.32 Methods

Three mature male New Zealand white rabbits were killed by pentobarbitone overdose and a transverse osteotomy was created in the right tibia via an anteromedial approach. In addition, the medullary cavity was reamed and nailed (see section 4.1), and a silastic sheath was wrapped around the osteotomy as previously described in section 4.2. By these methods, marrow and periosteal tissues were completely eliminated from the osteotomy sites. The blood supply was investigated by arteriography (see section 4.1) to provide baseline data for the recovery experiments.

In a parallel study, under general anaesthesia and using
aseptic techniques, a transverse osteotomy was created in the right tibia of each of 12 live mature male New Zealand rabbits. The marrow cavity was reamed and nailed as described in section 4.1, and a sheath was wrapped around the osteotomy as described in section 4.2. The animals were allowed to recover and were housed in standard pens and fed a standard diet. Groups of 3 animals were investigated one day, one week, two weeks and four weeks respectively after operation. Two animals were investigated by arteriography and the perfusion of the aorta was carried out under general anaesthesia (see section 4.1). In one animal the operated tibia was investigated by osteomedullography after death by pentobarbitone overdose (see section 4.1). Fracture healing was examined histologically in all cases (see section 4.1), sectioning being performed as in Fig. 51.

4.33 Results

All the animals survived for the planned duration of the experiment without distress or wound infection. Immediate post-operative radiographs revealed good alignment in all cases (Fig. 57). Radiological callus was not observed at the osteotomy site in any animal. Visual findings observed as the specimens were being obtained were similar to those reported in sections 4.1 and 4.2.

Post-decalcification micro-arteriographs obtained from the post-mortem experiments revealed the presence of intra-cortical vessels in both distal and proximal fragments (Fig. 58a). Some of these vessels were still perfused in the
recovery experiments 1 day after operation in the proximal but not in the distal fragments (Fig. 58b). The vessels were not perfused again in any fragments until 4 weeks after operation when some were perfused in the proximal but not in the distal fragments (Fig. 58c).

Micro-radiography after perfusion of the marrow revealed dye in the marrow cavities of both fracture fragments within the nail tracks. Therefore, as in section 4.1, this method of investigation was not found to provide any useful information.

Histological examination one day after operation revealed absent periosteal and marrow tissues in all cases (Fig. 59a). Macroscopic and histological examination of all excised specimens revealed non union. At one week, there was an endosteal reaction in the proximal but not in the distal fragment (Fig. 59b) in all cases. Proliferation of darkly staining round cells was observed in the endosteum in the proximal fragment (Fig. 59c). Elsewhere, fibrous/granulation tissue and bone formation were observed in the spaces between the nail track and the endosteal surface of the cortex. The distal fragment did not react at all in all cases (Fig. 59d). At 2 and at 4 weeks, the reaction in the proximal and necrosis in the distal fragment appeared to be more advanced (Fig. 59e). More mature bone could be observed in the marrow in the proximal fragment in the spaces between the nail track and the endosteal surface (Fig. 59f). The marrow in the distal fragment contained fibrous strands and no cells. The fracture gap was not bridged by intra-cortical healing or by endosteal callus in all specimens and a periosteal reaction was not observed in any
4.34 Discussion and conclusions

These experiments were also repeated for the reasons discussed in section 4.2 as the sheaths prevented penetration of the specimens by the fixative and the decalcifying agent.

The post-mortem study showed that some intra-cortical vessels may be preserved following procedures which damage both periosteal and nutrient blood supply. This vascular filling is presumably due to an intact epiphyseo-metaphyseal circulation and it probably accounts for a bone retaining its viability after simultaneous open reduction and reaming and nailing of fractures. However, of more importance is the fact that these vessels were not filled between one and four weeks after operation in the proximal fragments and during the entire duration of experiment in the distal fragment. The reasons for this are not clear but it is assumed that the vessels sustain damage in the post-operative period as a result of oedema. It is probable that re-vascularisation of the distal fragment would eventually occur if the period of observation had been long enough.

In this study, union has not been observed in the absence of periosteal and marrow tissues. However, it would appear that, as in section 4.2, the non union is due to avascularity particularly distal to the osteotomy site rather than simply because of tissue exclusion. The vascularised proximal fragment was shown to have a repair potential, presumably derived from
the endosteal membrane, despite being deprived of medullary and periosteal tissues. The commonest comparable clinical situation is the application of plastic cerlage bands to shaft fractures around cemented intramedullary implants. This study clearly shows that this practice is fraught with the danger of delayed healing due in part to a poor blood supply to the fracture fragments.
Figure 57
Radiograph of an osteotomised and sheathed rabbit tibia, showing site of osteotomy (arrowed), silastic sheath held in place by cerclage wires, and intramedullary nail

Figure 58a
Micro-arteriograph (post-decalcification) of a longitudinal section through a nailed and sheathed osteotomy created after death, showing perfusion of cortical vessels in both fragments
Figure 58b
Micro-arteriograph (post-decalcification) of a longitudinal section through a nailed and sheathed osteotomv created in a live animal 1 day after operation, showing lack of significant vascular perfusion in fragments.

Figure 58c
Micro-arteriograph (post-decalcification) of a longitudinal section through a nailed and sheathed osteotomy created in a live animal 4 weeks after operation, showing return of vascular perfusion in the proximal fragments.
**Figure 59a** Photo-micrograph (H & E) of a longitudinal section through a nailed and sheathed osteotomy in a live animal 1 day after operation, showing that most of the marrow has been removed (mag x 5)

**Figure 59b** Photo-micrograph (H & E) of a longitudinal section through a nailed and sheathed osteotomy in a live animal 1 week after operation, showing an endosteal reaction in the proximal but not in the distal fragment (mag x 5)
Figure 59c  Photo-micrograph (H & E) of a higher magnification of the endosteal reaction in the proximal fragment seen in Figure 59b (mag x 25)

Figure 59d  Photo-micrograph (H & E) of a higher magnification of the area of non reacting endosteum of the distal fragment seen in Figure 59b (mag x 25)
Figure 59e Photo-micrograph (H & E) of a longitudinal section through a nailed and sheathed osteotomy in a live animal 4 weeks after operation, showing a more advanced endosteal reaction in the proximal but not the distal fragment (mag x 5)

Figure 59f Photo-micrograph (H & E) of a higher magnification of the proximal fragment seen in Figure 59e, showing cartilage (c) and new bone formation (B) (mag x 25)
Figure 59g  Photo-micrograph (H & E) of a higher magnification of the distal fragment seen in Figure 59e, showing non cellular fibrous strands in the marrow (mag x 25)
4.4 **SUMMARY**

The relative contribution of periosteum, marrow and intra-cortical tissues to the healing of an experimental fracture of the rabbit tibia has been studied by selective tissue exclusion techniques. Bone marrow tissue was excluded by reaming and nailing and the periosteum was excluded by the application of a silastic sheath around the shaft. Non union was observed in all cases where the periosteum was excluded from the fracture site but not when the marrow alone was excluded. Thus, the periosteum appears to be the critical tissue which determines whether a tibial shaft fracture heals or not. However, in the absence of the periosteum, the marrow was shown to be capable of forming bone provided there was a continuing medullary blood supply. The endosteum membrane was also shown to be capable of forming bone, in the absence of both marrow and periosteal tissues, provided the fracture fragment was vascularised.
5.0 EXPERIMENTAL STUDIES OF BLOOD SUPPLY
5.1 AN INVESTIGATION OF THE PERIOSTEAL CONTRIBUTION TO THE
BLOOD SUPPLY OF AN ISOLATED DIAPHYSEAL SEGMENT OF THE MATURE
RABBIT TIBIA.

5.11 Introduction

A precise knowledge of the important portals of entry of blood to fracture fragments is vital to our understanding of delayed union. There are three possible routes; namely, extra-osseous (including periosteal), intracortical and nutrient (medullary) but the relative importance of each to fracture healing is not known with any certainty. As discussed in the introduction to this thesis (section 1.2), some authors favour the periosteal blood supply (Kolodny 1923, Cavadias & Trueta 1965) and others favour the nutrient blood supply (Ladanyi & Hidvegi 1954, Rhinelander & Baragry 1962). Furthermore, although it has not been shown that damage to blood supply definitely leads to delayed union, most orthopaedic surgeons believe it to be an important factor (Watson Jones 1943, Wilson 1981). If this were so, then the circulation most important for healing must be severely damaged in slowly healing fractures.

It is customary to investigate the routes of blood supply to the diaphyseal cortex by vascular occlusion techniques (Johnson 1927, Foster et al. 1951, Trueta & colleagues 1955, 1964, 1965, 1968). However, as discussed in the introduction to this thesis (section 1.2), most vascular occlusion techniques used previously do not successfully isolate individual
components completely. Consequently, the present study has explored new models, in which a segment of the tibial diaphysis has been isolated, for investigating individual contributions to the blood supply of bone (Fig. 60).

A transverse osteotomy of the shaft of a long bone should interrupt both nutrient and proximal epiphyseo-metaphyseal vascular connections to the distal fragment if carried out distal to the point of entry of the nutrient artery. Therefore, a double osteotomy should effectively deprive the diaphyseal segment so produced of both nutrient and epiphyseo-metaphyseal (proximal and distal) blood supply. It is technically possible to preserve most of the periosteal connections to this segment by limiting soft tissue dissection to the osteotomy sites. The nutrient artery may then be prevented from reforming by the introduction of an intramedullary nail. Thus, this model could provide an opportunity to examine, in isolation, the periosteal contribution to the blood supply to the diaphyseal cortical segment.

5.12 Method

Three mature male New Zealand white rabbits were killed by pentobarbitone overdose and a 2cm long diaphyseal segment was created in each tibia. In addition, the medullary cavity of the right tibia was reamed and nailed. This procedure completely interrupts the nutrient and epiphyseo-metaphyseal blood supply. The soft tissue attachments were carefully preserved in order to retain the periosteal circulation. The blood supply of the diaphyseal segment was investigated by
arteriography and the cortex was examined histologically as previously described in section 4.1 to provide baseline data for recovery experiments.

In a parallel study, under general anaesthesia and using aseptic techniques, a 2cm long diaphyseal segment was created, reamed and nailed in the right tibia of each of 12 live mature male New Zealand rabbits as above. The animals were allowed to recover and were housed in standard pens and fed a standard diet. Groups of 3 animals were investigated one day, one week, two weeks and four weeks respectively after operation. Two animals were investigated by arteriography perfusing the aorta under general anaesthesia (see section 4.1). In one animal, the operated tibia was investigated by osteomedullography after death by pentobarbitone overdose (see section 4.1). The cortex of the isolated diaphyseal segment was examined histologically in all cases for evidence of necrosis.

Choice of experimental animals:

The rabbit was chosen as the experimental animal because the blood supply to the diaphysis of its long bones is well known and has been shown by several workers to be similar to that of man (Trueta 1968, Brookes 1971, Nagi 1981). Only mature male animals as defined previously in section 4.0 were used to reduce the influence of constitutional factors on the results. In addition, the bones of male animals were found to be less brittle and, therefore, technically easier to handle.

The observation times were selected to coincide with the
investigation on fracture healing and post-mortem studies were carried out to provide baseline data for the recovery experiments.

Operative procedures:

Anaesthesia was as previously described in section 4.0 using midazolam ("Hypnovel", Roche), as premedication, and a nitrous oxide-oxygen-halothane mixture by face mask for maintenance of anaesthesia during surgery. Standard aseptic surgical techniques were employed in all survival experiments and the right tibia only was operated on in all cases.

The operation site was prepared and sterilised with povidone iodine solution ("Betadine surgical scrub", NAPP Laboratories) and with chlorhexidine gluconate ("Hibitane", ICI). The subcutaneous border of the middle third of the tibia was exposed through an anteromedial incision. The skin edges and the muscles were retracted away from the shaft at the proposed osteotomy sites by inserting McDonald’s dissectors. Soft tissue attachments to the shaft elsewhere, including the periosteum, were preserved. A transverse osteotomy was created at the proximal site using a fine-toothed, air-powered reciprocating/sagittal saw (Zimmer Micro 100). The saw blade was 100u thick to reduce thermal burns to the bone and bone cutting was carried out under continuous normal saline irrigation. The medullary cavity was reamed proximally and distally from the osteotomy site. An opening for the insertion of the nail was obtained by allowing the reamer to extrude proximally through a second wound over the anterior tibia. The
fragments were reduced and the nail (a 10cm long, 1mm thick Kirschner wire or 2mm thick Steinmann's pin) was introduced a few mm past the osteotomy site. Next a second osteotomy was created 2cm distal to the first and the nail was then fully introduced into the medullary cavity.

Duplocillin 0.25ml (75mg procaine penicillin and 56.25mg benzathine penicillin, Gist-Brocades) was infiltrated into the wound locally before closure. The wound was closed in layers with interrupted 4/0 coated vicryl ("polyglactin 910", Ethicon) to the deep fascia and continuous 3/0 supramid ("white, pseudo-monofilament polyamide", BBM-AG) to the skin.

Post-operative care, perfusion techniques and preparation of specimens for micro-radiography and for histology were as described elsewhere in section 4.1 except that transverse sections were obtained instead of longitudinal sections as shown in Fig. 61. As suggested by previous workers, notably Trueta (1968) and Sevitt (1981), the absence of osteocytes from the cortical lacunae was assumed to indicate lack of blood supply.

5.13 Results

All animals survived for the planned duration of experiments. The operation wounds healed within a few days and there were no wound infections. A number of the operated tibiae initially showed some rotational movement but this ceased within a few days.
Radiographic examination immediately after operation revealed good alignment in all cases (Fig. 62a). Plain radiographs at 2 and at 4 weeks after operation revealed prominent external calluses in relation to each osteotomy site and the diaphyseal segment was united to the shaft (Fig. 62b).

Visual inspection of operated legs one day after operation and at the time of death revealed that extensive bleeding had occurred into the soft tissues far beyond the vicinity of the osteotomies. At one week, the tissues were extensively discoloured and there was extensive interstitial oedema and tissue swelling. At two and at four weeks, these tissue reactions had subsided and the soft tissues were adherent to the osteotomy sites. A diffuse thickening was often palpable in relation to each osteotomy site extending 1 or 2cm proximally and distally.

In the post-mortem studies, post-decalcification micro-arteriographs of the diaphyseal segment showed vessels penetrating the cortex from its outer surfaces in profuse numbers (Fig. 63a). The perfused dye also penetrated the marrow but no recognisable vessels were demonstrated. Few vessels were perfused in the cortex after the segment was reamed and nailed (Fig. 63b).

In the recovery experiments, micro-arteriographs obtained after decalcification revealed absent cortical vessels in day-old and week-old segments (Fig. 63c). At 2 and at 4 weeks respectively, periosteal afferent vessels were observed to have returned and to penetrate the cortex centripetally (Fig. 63d).
Perfused blood vessels were also observed at 4 weeks in the medullary cavity in the spaces between the nail and the cortex.

Micro-radiography after injecting dye into the marrow cavity revealed that the perfusate penetrated the isolated segment through the nail track in all cases. Thus, as in section 4.0, this method of investigation did not provide any useful information.

Histological sections of diaphyseal segments created and nailed post mortem and in live animals 1 day after operation revealed that most of the marrow had been removed at operation (Fig. 64a). Examination of diaphyseal segments up to one week old revealed essentially normal cortex with most of the lacunae occupied by osteocytes (Fig. 64b). At 1 week, there was in addition, sub-periosteal new bone, as well as cartilage, formation over the entire circumference of the shaft (Figs. 64c & 64d). At 2 and at 4 weeks, there was similar periosteal reaction as above (Fig. 64e). In addition, there was evidence of significant osteocyte loss from the cortex which followed no specific, consistent or anatomical pattern (Fig. 64f).

5.14 Discussion and conclusions

This study has attempted to demonstrate periosteal contribution to diaphyseal blood supply. The post-mortem perfusion study was performed in order to demonstrate the possible routes of supply before they could be interfered with by patho-physiological changes. This baseline study has been omitted by previous workers and it shows periosteal afferent
proposed by Wray (1963). Nevertheless, if one extrapolates these findings to man, it may explain why Gregg et al. (1983, 1984, 1986) were able to demonstrate "cold spots" in the early stages after fracture but found no correlation between "cold spots" and delayed union. Therefore, a period of time must be allowed to elapse before assessing permanent vascular damage sustained in a fracture by bone scintigraphy.

This study does not confirm the view of cortical vascularisation which identifies discrete territories of blood supply. Osteocyte loss, where it occurred, was patchy and was not neatly separable into an endosteal sector as described by Trueta and his colleagues (1968). This patchy pattern of osteocyte loss may result from, as suggested by Danckwardt-Lilliestrom (1969), the filling in or obturation of the endosteal openings of cortical vascular channels, by marrow debris and cortical dust produced by reaming.

Although afferent vessels have been demonstrated in this study by perfusion techniques, it does not guarantee that in vivo they are capable of providing adequate blood supply. However, on the basis of cortical osteocyte loss, it would appear that the periosteal circulation is capable of at least maintaining many osteocytes. The actual blood supply may have been better measured by the microsphere method but the aim of the present experiment was to study the routes of blood supply.

This study may further be criticized on the grounds that only micro-radiographs of transverse sections were examined. Thus, contributions by longitudinally running medullary
vessels to the re-vascularisation of the cortex of the isolated
diaphyseal segment could have been missed. This is unlikely,
however, because a longitudinally running vessel, if present,
would have been observed in each successive section examined.
This was not the case in any specimen and, therefore, the
conclusion that the periosteal circulation was entirely
responsible is justified.
Figure 60a  Diagram showing a new experimental model for investigating the blood supply of the diaphyseal cortex
36 NZW rabbits

12 gpI

12 gpII

12 gpIII

intramedullary

nailing

combination

extra-

periosteal

sheath

3 animals killed at
Day 1, Weeks 1, 2, & 4

arteriography

osteomedullary

2 animals

1 animal

microangiography

histology

Figure 60b Flow diagram of experimental procedures for investigating cortical blood supply
for histology sections  3 x 1 mm thick slices for X-rays

Figure 61  Diagram of schema for sections
Figure 62a
Radiograph of a nailed diaphyseal segment of the rabbit tibia, showing site of osteotomy (arrowed) and intramedullary nail

Figure 62b
Radiograph of a nailed diaphyseal segment created in a live animal 4 weeks after operation, showing fracture callus (arrowed) in relation to each osteotomy site
Figure 63a
Micro-arteriograph (post-decalcification) of a transverse section through a diaphyseal segment created after death, showing vessels penetrating the cortex from its outer surfaces in profuse numbers.

Figure 63b
Micro-arteriograph (post-decalcification) of a transverse section through a reamed and nailed diaphyseal segment created after death, showing fewer vessels perfused.
Figure 63c
Micro-arteriograph (post-decalcification) of a transverse section through a reamed and nailed diaphyseal segment created in a live animal 1 week after operation, showing absence of vessels.

Figure 63d
Micro-arteriograph (post-decalcification) of a transverse section through a reamed and nailed diaphyseal segment in a live animal 4 weeks after operation, showing the return of periosteal, cortical and marrow vessels.
Figure 64a  Photo-micrograph (H & E) of a transverse section through a reamed and nailed diaphyseal segment created in a live animal 1 day after operation (mag x 5)

Figure 64b  Photo-micrograph (H & E) of a higher magnification of the cortex seen in Figure 64a, showing most of the lacunae occupied by osteocytes (mag x 25)
Figure 64c  Photo-micrograph (H & E) of a transverse section through a reamed and nailed diaphyseal segment created in a live animal 1 week after operation, showing subperiosteal new bone formation (B) (mag x 5)

Figure 64d  Photo-micrograph (H & E) of a higher magnification of the cortex seen in Figure 64c, showing subperiosteal new bone formation (B), cartilage formation (C) and empty lacunae (EL) (mag x 25)
Figure 64e  Photo-micrograph (H & E) of a transverse section through a reamed and nailed diaphyseal segment created in a live animal 4 weeks after operation (mag x 5)

Figure 64f  Photo-micrograph (H & E) of a higher magnification of the cortex seen in Figure 64e, showing patchy incomplete osteocyte loss from lacunae (mag x 25)
5.2 AN INVESTIGATION OF THE NUTRIENT ARTERY CONTRIBUTION TO
THE BLOOD SUPPLY OF AN ISOLATED DIAPHYSEAL SEGMENT OF THE
MATURE RABBIT TIBIA.

5.21 Introduction

Nutrient artery connections to a segment of the diaphysis may be preserved by the fracture technique described by Ashurst and her colleagues (1982). The shaft is fractured at two levels by osteoclasis rather than by osteotomy to interrupt only the longitudinally running intracortical vessels. In theory, the main trunks of the nutrient artery should remain intact as suggested by Fig. 60. The periosteal supply to the segment may then be obstructed by interposing a non-porous material between the soft tissues and the shaft. As a result, both periosteal and longitudinally running epiphyseo-metaphyseal vascular connections to the segment are interrupted and the blood supply totally entrusted to the nutrient circulation.

This model assumes that there are two potential routes of venous escape from the cortex; periosteal and medullary. In most accounts of the osseous circulation, some medullary vessels are regarded as afferent to the cortex and others as efferent (Brookes 1971, Rhinelander 1972, 1974) perhaps discharging ultimately into a central venous sinus and, thereafter, into the nutrient veins. Thus, in theory, there is a readily available medullary route for venous escape from the cortex in the event that the periosteal route is blocked.
In this study, the blood supply of the diaphyseal cortex has been examined following the interruption of periosteal and epiphyseo-metaphyseal vascular connections to an isolated diaphyseal segment.

5.2.2 Methods

Three mature male New Zealand white rabbits were killed by pentobarbitone overdose and a 2cm long diaphyseal segment was created in the right tibia by osteoclasis as follows: The subcutaneous border of the middle third of the tibia was exposed through an anteromedial incision. The skin edges and the muscles were retracted away from the shaft and the periosteum was stripped away from its middle third. A 3 cm long semi-rigid silastic tube was cut open length-wise and placed around the shaft and two McDonald's dissectors were introduced between it and the shaft. The sites for the two osteotomies were marked 2cm apart and scored circumferentially using a fine-toothed, air-powered reciprocating/sagittal saw (Zimmer Micro 100). The saw blade was 100μ thick to reduce thermal burns to the bone and bone cutting was carried out under continuous normal saline irrigation. The dissectors were removed and the sheath was fastened on to the shaft with four cerclage wires; two over the diaphyseal segment so created and one at each end of the sheath to prevent ingrowth of vessels through gaps between the sheath and the shaft. The osteotomies were then completed by osteoclasis using a specially adapted Lohman’s clamp.

Thus, the epiphyseo-metaphyseal connections were
interrupted by osteoclasis, the periosteal circulation was excluded by application of a sheath and the nutrient artery blood supply was completely isolated. To confirm this and to provide baseline data for the recovery experiments, the blood supply of the isolated segment was investigated by arteriography and the cortex was also examined histologically (see section 4.1).

In a parallel study, under general anaesthesia and using aseptic techniques, the isolated diaphyseal segment was created in the right tibia of each of 12 live mature male New Zealand rabbits by application of a silastic sheath and by osteoclasis as above. A plaster of Paris cast was applied to provide additional support. The animals were allowed to recover and were housed in standard pens and fed a standard diet. Groups of 3 animals were investigated one day, one week, two weeks and four weeks respectively after operation. Two animals were investigated by arteriography perfusing the aorta under general anaesthesia (see section 4.1). In one animal the operated tibia was investigated by osteomedullography after death by pentobarbitone overdose (see section 4.1). The cortex of the diaphyseal segment was examined histologically in all cases for evidence of necrosis, sectioning being performed as in Fig. 61.

5.23 Results

All the animals survived the planned duration of experiment without distress or wound infection.

Radiographic examination immediately after operation
(Fig. 65) revealed a small degree of angulation in some cases. No radiological callus formation was observed in any of the operated tibiae and none of the osteotomies were united with the shaft. Initially, haemorrhagic/inflammatory reaction similar to that described in section 5.1 was observed and this subsided by Week 2.

In the post-mortem studies, post-decalcification micro-arteriography revealed some branches of the nutrient artery penetrating the cortex centrifugally (Fig. 66a). These vessels were still perfused at 1 day and at 1 week after operation. At 2 weeks, marrow afferent vessels were perfused but generally the cortical afferent vessels were not (Fig. 66b). Both cortical and medullary afferent vessels were no longer perfused 4 weeks after operation (Fig. 66c).

Micro-radiography after perfusion of marrow revealed the presence of dye in the marrow cavity and in cortical efferent vessels at 1 day and at 1 week (Fig. 66d). The segment was not penetrated by injected dye at 2 and at 4 weeks after operation.

Histological sections revealed a normal cortex, with most of the lacunae occupied by osteocytes, and a normal marrow at Day 1 and at Week 1 (Figs. 67a & 67b). At 2 weeks, there was evidence of patchy osteocyte loss and these became more extensive at 4 weeks (Figs. 67c and 67d). New bone formation was not observed on the external surface of the isolated segment or within its marrow cavity.
5.24 Discussion and conclusions

These studies were repeated for the reasons given in section 4.2. The sheaths prevented the penetration of the initial lot of specimens by the fixative and decalcifying agent.

In this investigation, an attempt has been made to isolate for study the nutrient artery (medullary) circulation. The post-mortem arterial perfusion study, which revealed routes of supply before they could be affected by pathophysiological changes, showed the longitudinally running afferent vessels of the marrow and their transverse branches which penetrated the cortex centrifugally. This suggests that the technique of osteoclasis must have preserved the afferent arm of the nutrient artery (medullary) circulation as intended.

However, cortical perfusion, demonstrated post mortem and at Day 1, gradually diminished with time in these circumstances in which the silastic sheath and osteoclasis have eliminated periosteal and longitudinally running intra-cortical connections to the isolated diaphyseal segment. Furthermore, the vessels were not perfused at all 4 weeks after operation. The reasons for this are not clear but may be related to the effect of post-operative oedema or additional damage caused by motion at the osteotomy sites as the animals mobilised. The use of an external fixator could have avoided the latter but this would have introduced another variable and made the interpretation of results even more difficult.
The absence of an effective blood supply presumably explains why no cellular reaction was observed within the marrow cavity of the diaphyseal segment. In addition, cortical osteocyte loss increased with time. This confirms the perfusion findings which showed cortical perfusion progressively diminishing with time. However, many osteocytes were still evident in their lacunae even at 4 weeks which suggests a continuing blood supply not detectable by the perfusion technique or, alternatively, and more likely, an inherent resistance of some cortical osteocytes to ischaemia (see section 5.1). The surviving osteocytes were located haphazardly in the cortex, in the areas bordering the periosteum and endosteum and in the depth of the cortex. Therefore, as in section 5.1, this study does not confirm the view of cortical vascularisation which identifies discrete territories of blood supply.
Figure 65
Radiograph of a sheathed diaphyseal segment, showing sites of osteotomy (arrowed) and silastic sheath held in place with cerclage wires.

Figure 66a
Micro-arteriograph (post-decalcification) of a transverse section through a sheathed diaphyseal segmented created after death, showing vessels within the marrow and penetrating the cortex from its endosteal surfaces in profuse numbers.
Figure 66b
Micro-arteriograph (post-decalcification) of a transverse section through a sheathed diaphyseal segment created in a live animal 2 weeks after operation, showing perfusion of marrow afferent vessels but generally the cortical afferent vessels were not perfused.

Figure 66c
Micro-arteriograph (post-decalcification) of a transverse section through a sheathed diaphyseal segment created in a live animal 4 weeks after operation, showing absence of perfusion in the marrow and cortex.
Figure 66d  Micro-radiography (post-decalcification) after perfusion of marrow of a transverse section through a sheathed diaphyseal segment created in a live animal 1 day after operation, showing dye within the marrow and some transverse cortical efferent vessels.
**Figure 67a** Photo-micrograph (H & E) of a transverse section through a sheathed diaphyseal segment created in a live animal 1 day after operation (mag x 5)

![Image of Figure 67a](image)

**Figure 67b** Photo-micrograph (H & E) of a higher magnification of the cortex and marrow seen in Figure 67a, showing most of the lacunae to be occupied by osteocytes (mag x 25)

![Image of Figure 67b](image)
Figure 67c  Photo-micrograph (H & E) of a transverse section through a sheathed diaphyseal segment created in a live animal 4 weeks after operation (mag x 5)

Figure 67d  Photo-micrograph (H & E) of a higher magnification of the cortex seen in Figure 67c, showing extensive cortical osteocyte loss (mag x 25)
5.3 AN INVESTIGATION OF THE EPiphySEO-METAPHYSEAL CONTRIBUTION TO THE BLOOD SUPPLY OF AN ISOLATED DIAPHYSEAL SEGMENT OF THE MATURE RABBIT Tibia.

5.3.1 Introduction

Of the three osseous circulatory systems, the contribution of the epiphyseo-metaphyseal to diaphyseal blood supply is the most obscure. Trueta and Cavadas (1964) have attempted to isolate the epiphyseo-metaphyseal circulation and observe its contribution to cortical blood supply but their results were inconsistent. In some animals in their series no changes were observed but in others there was total necrosis of cortex and marrow. This may be due in part to their experimental model as discussed in the introduction to this thesis (section 1.2). Therefore, a gap still exists in our knowledge of the contribution of this circulation to diaphyseal blood supply.

The question arises as to whether the longitudinally running intracortical (haversian) vessels anastomose with the epiphyseo-metaphyseal circulation. The perfusion findings in section 4.3 in relation to a transverse osteotomy, in which the periosteal and medullary tissues had been deliberately excluded, suggest that they do. Therefore, this circulation could provide a possible route of blood supply to a portion of the diaphysis deprived of both the periosteal and the medullary blood supply. This latter information, if available, would be of importance to orthopaedic surgeons because of the need, in
certain circumstances, to apply Patridge and other cerclage bands to shaft fractures in the presence of a medullary cavity occupied by the stem of a prosthesis and bone cement.

It is for these reasons that the following study was carried out and it examines the potential epiphyseo-metaphyseal contribution to the re-vascularisation of an isolated diaphyseal segment model (Fig. 60).

5.32 Methods

Three mature male New Zealand white rabbits were killed by pentobarbitone overdose and a 2cm long diaphyseal segment was created in the right tibia via an anteromedial approach using two transverse osteotomies. In addition, the tibia was reamed and nailed using a retrograde technique as described previously in section 5.1 and a silastic sheath was wrapped around the shaft as described in section 5.2. These procedures completely interrupt the nutrient and periosteal circulations and entrust the re-vascularisation of the isolated diaphyseal segment to the epiphyseo-metaphyseal blood supply. The blood supply of the segment was investigated by arteriography (see section 4.1) and the cortex was examined histologically to provide baseline data for the recovery experiments, sectioning being carried out as in Fig. 61.

In a parallel study, under general anaesthesia and using aseptic techniques, a 2cm long diaphyseal segment was created in the right tibia of each of 12 live mature male New Zealand rabbits. The tibia was also reamed and nailed and a silastic
sheath was wrapped around the shaft as above. The animals were allowed to recover and were housed in standard pens and fed a standard diet. Groups of 3 animals were investigated one day, one week, two weeks and four weeks respectively after operation. Two animals were investigated by arteriography perfusing the aorta under general anaesthesia (see section 4.1). In the third animal, the tibia was investigated by osteomedullography after death (see section 4.1). The cortex of the diaphyseal segment was examined histologically in all cases for evidence of necrosis, sectioning being carried out as in Fig. 61.

5.33 Results

The animals tolerated the procedures well and there was no post-operative wound infection.

Radiographic examination revealed good alignment in all cases post-operatively (Fig. 68). No callus formation was observed in any of the operated tibiae and no osteotomy healed with the shaft.

No cortical or medullary vessels were perfused in the post-decalcification micro-arteriographs obtained from both post-mortem and live experiments. Dye was observed in the marrow cavity and on the external surface of segments created after death (Fig. 69a) and 1 day post-operatively in live experiments (Fig. 69b) but not 1, 2 or 4 weeks after operation (Fig. 69c).
Micro-radiography after marrow perfusion revealed presence of dye in the nail track within the marrow in all cases. No vessels were demonstrated and, therefore, this method of investigation was not found to provide any useful information.

Histological sections revealed a normal cortex with most of the lacunae occupied by osteocytes at Day 1 and Week 1 after operation (Figs. 70a & 70b). At 2 weeks, there was some significant loss of osteocytes in the endosteal and periosteal sectors. At 4 weeks, most of the cortex had become devoid of osteocytes but there were still a number of lacunae occupied by osteocytes (Figs. 70c & 70d). New bone formation was not observed on the external surface of the isolated segment or within its marrow.

5.34 Discussion and conclusions

These experiments were also repeated for the reasons given in section 4.2. The sheaths did not permit the penetration of the initial specimens by fixing and decalcifying agents.

In this attempt to isolate the epiphyseal-metaphyseal circulation for study, post-mortem perfusion revealed that the application of a nail and sheath completely destroyed both medullary and cortical vessels in the isolated diaphyseal segment.

The recovery study revealed that re-vascularisation had
not occurred at 4 weeks. This suggests that the longitudinally running intra-cortical vessels could not by themselves re-vascularise an isolated diaphyseal segment as claimed by Olerud and Danckwardt-Lilliestrom (1971). These authors demonstrated that a diaphyseal segment could be successfully re-vascularised and united with the rest of the fractured bone after compression plating. They showed that healing was by the cutter-head mechanism but this occurred in association with the re-establishment of the medullary circulation. Olerud and Danckwardt-Lilliestrom (1971) did not eliminate the medullary circulation in their experiment and this was probably a source of the cutter-heads observed in the cortex by these authors. In the present study, both medullary and periosteal circulations which could have given rise to similar cutter-heads have been destroyed. These results suggest that application of Patridge and other bands to fractures around prosthetic stems should not be encouraged.

Normal looking osteocytes were observed at 4 weeks in the recovery experiments although it is unlikely that the diaphyseal segment was receiving a blood supply. This confirms the views expressed in section 5.1 that some osteocytes may be resistant to ischaemia. It, therefore, raises the possibility that there is no direct correlation between osteocyte loss and loss of blood supply. However, it seems likely that osteocytes deprived of a blood supply for long enough will eventually die.

The present study could be criticized on two counts. First, proximal and distal reaming could have damaged the cortical vessels, through which re-vascularisation might have
taken place, for the reasons discussed in section 5.1, where even in the presence of the periosteal circulation, the cortical vessels did not fill at Day 1 and Week 1 after operation. But they did fill again from two weeks onwards. It is possible, but unlikely, that the use of suction methods to remove the marrow contents, could have altered the results. Second, 4 weeks may be too short an observation period in which to demonstrate the ability of intra-cortical vessels to penetrate an isolated diaphyseal segment. Further study of this is required by extending the observation time in the recovery experiment.
Figure 68
Radiograph of a nailed and sheathed diaphyseal segment, showing sites of osteotomy (arrowed), silastic sheath held in place with cerclage wires, and intra-medullary nail

Figure 69a
Micro-arteriograph (post-decalcification) of a transverse section through a nailed and sheathed diaphyseal segment created after death, showing a general lack of perfusion of cortical vessels
Figure 69b
Micro-arteriograph (post-decalcification) of a transverse section through a nailed and sheathed diaphyseal segment created in a live animal 1 day after operation, showing a general lack of perfusion of cortical vessels.

Figure 69c
Micro-arteriograph (post-decalcification) of a transverse section through a nailed and sheathed diaphyseal segment created in a live animal 4 weeks after operation, showing a general lack of perfusion of cortical vessels.
Figure 70a  Photo-micrograph (H & E) of a transverse section through a nailed and sheathed diaphyseal segment created in a live animal 1 day after operation (mag x 5)

Figure 70b  Photo-micrograph (H & E) of a higher magnification of the cortex seen in Figure 70a, showing most of the lacunae occupied by osteocytes (mag x 25)
Figure 70c  Photo-micrograph (H & E) of a transverse section through a nailed and sheathed diaphyseal segment created in a live animal 2 weeks after operation (mag x 5)

Figure 70d  Photo-micrograph (H & E) of a higher magnification of the cortex seen in Figure 70c, showing extensive cortical osteocyte loss (mag x 25)
Attempts to isolate completely the periosteal, nutrient (medullary) and epiphyseo-metaphyseal circulations have been made in this study using a diaphyseal segment model. The periosteal circulation was isolated by creating and nailing a diaphyseal segment. The medullary circulation was isolated by creating and ensheathing a diaphyseal segment. The epiphyseo-metaphyseal circulation was isolated by combining these two techniques. Cortical arterial perfusion was observed in diaphyseal segments from 1 day to 2 weeks but not afterwards following the isolation of the nutrient circulation. Whereas, cortical arterial perfusion was not observed from 1 day to 1 week but afterwards following the isolation of the periosteal circulation and the cortex remained well perfused at 4 weeks. There was no perfusion of cortical vessels in the diaphyseal segment with the isolation of the epiphyseo-metaphyseal circulation. Thus, it would appear that an intact periosteal circulation is necessary, as demonstrated by arterial perfusion techniques, if an isolated segment of cortical bone is to continue to be adequately perfused. However, some osteocytes in the cortex of the isolated diaphyseal segment survived four weeks after each isolation procedure. This may be due in part to the fact that loss of staining ability takes some time to manifest after osteocyte death.
6.0 GENERAL DISCUSSION AND CONCLUSIONS
Closed fractures of the adult tibial shaft are common and potentially serious injuries which often result in considerable morbidity. One reason for this is the slow healing displayed by many of these fractures. Unfortunately, the reasons for this are not known and, therefore, prevention is not possible. This is also a difficult area to investigate because there are so many variables involved. In this study, the problem has been re-examined, beginning with a clinical study and then examining aspects which arose from this in the laboratory.

In order to define the extent of the problem in a more homogeneous fracture population, the natural history of 100 closed unilateral fractures treated in a standard fashion by closed methods was examined in a prospective study. The true delayed healing rate was determined and the clinical factors that may be important in slow healing were investigated. In addition, serum biochemical changes were investigated over a 20 week period following fracture and an attempt was made to use sequential bone scintigraphy to predict which fractures would not heal on time.

The clinical study revealed that there was a delayed healing of the fracture beyond 20 weeks in 19% of the patients. However, of interest was that 15 of the 19 cases of delayed union united satisfactorily with a further period of 10 weeks conservative treatment. No relationship was found between age, sex, fracture site and morphology, accompanying intact fibula and time to union. The only statistically
significant clinical factor related to delayed healing was high energy violence.

The serum level of creatinine phosphokinase (CPK), a muscle enzyme, was found to rise sharply in most cases within hours after fracture. The peak levels reached were significantly higher after high energy violence compared with low energy violence ($p=0.001$). The peak levels reached in delayed union were also higher compared with normal union but this was not statistically significant. The serum levels of inorganic phosphate and calcium also rose sharply after fracture but always remained within the reference range. The mean values reached a peak at 2 weeks and, thereafter, progressively fell. This progressive fall was more rapid in cases of delayed union and reached statistical significance at Week 16 for inorganic phosphate ($p=0.020$) and Week 14 for calcium ($p=0.053$). Unlike inorganic phosphate and calcium levels, no clearcut elevation could be observed from the baseline levels of osteocalcin, a non-collagenous bone specific protein secreted by osteoblasts. However, the mean value for patients with normal healing, compared with patients with delayed healing, was higher at each interval of measurement but this was only significantly different at Week 16 ($p=0.001$). There were no differences in the serum somatomedin activity after fracture for patients with normally uniting compared with patients with slowly uniting fractures.

All the recent methods of scintigraphic evaluation of fracture healing were re-examined but only one method was found to be of potential value. Sequential static scintigraphy
revealed that, at 6 weeks after fracture, the A/C ratio of uptake at the fracture site relative to an adjacent site on the fractured tibia was greater than 2.0 for normally uniting fractures and less than 2.0 for delayed union (p=0.00). This cut off value is different from that reported from elsewhere (Smith et al. 1987).

It was concluded from the clinical studies that:

1. more than 95% of closed adult tibial shaft fractures would unite within 30 weeks when treated conservatively in plaster casts and this provides baseline data against which other treatment methods may be compared;

2. measurement of CPK levels in the serum in the immediate post-fracture period may provide some indication of severity of trauma and, thereby, of prognosis;

3. normal union is different from delayed union with regards to changes in the serum levels of calcium, inorganic phosphate and osteocalcin during healing but these changes are unlikely to be clinically useful prognostic indicators; and that

4. examination of the A/C ratio on a static scintigram (using $^{99m}$Tc MDP) 6 weeks after fracture is of potential value with regards to predicting union but routine use of bone scintigraphy to predict delayed union is not straightforward and may require that each centre initially establish its own reference A/C values.
Since the clinical studies suggested that high energy violence was of prognostic importance, the next question posed was what effect high energy violence had on bone? In the clinical study (see section 2.1), high energy violence was associated predominantly with transverse and oblique fractures and low energy violence with spiral fractures as has been observed by other workers (Ellis 1958a, Bauer et al. 1962, Hoaglund & States 1967, Allum & Howbray 1980). Therefore, in the laboratory, different fracture types were produced manually and mechanically and the associated forces and patterns of soft tissue damage were investigated in dead animals.

It was found that, for any given force, less was required to produce transverse than spiral fractures and, therefore, more remained to be expended in the soft tissues and produce further damage. Furthermore, spiral fractures resulted in longitudinal lacerations of the periosteum and incomplete damage to the marrow. Whereas, transverse fractures produced circumferential lacerations of the periosteum with widespread stripping from the fracture site and complete transection of the marrow. Transverse fractures interrupted the continuity of the main trunks of the nutrient artery at the site of fracture but the stumps of the divided vessels distal to the fracture were still demonstrated by arterial perfusion.

It was concluded from these preliminary laboratory investigations that:
1. different forces produce different fracture types and these are associated with different patterns of soft tissue damage;

2. transverse fractures may be associated with the greater damage to the soft tissues and to the marrow structures compared to spiral fracture; and, that

3. although the nutrient artery may be interrupted by fractures, perfusion of the main trunks on either side of, and up to, the fracture line may still be observed because of available collateral channels.

Thus, the clinical and the preliminary laboratory studies suggested a possible link between delayed healing, high energy violence and its effects on the soft tissues but the exact way in which this leads to delayed union require further study. Therefore, an attempt was made to investigate which of the soft tissues or osseous circulations was the most important in fracture healing.

A fracture model was created by making a transverse osteotomy in the tibial diaphysis of mature rabbits. The periosteum, marrow and both respectively were excluded from the fracture site. The animals were allowed to recover and were then killed at intervals from 1 day to 4 weeks. It was found that only the exclusion of the periosteal tissues from the fracture site lead to non union at 4 weeks. The osteotomy of the rabbit tibia healed within the normal 2 to 4 week period after operation when the medullary tissues were excluded. As expected, when both were excluded non union was
also present at 4 weeks.

In another group of mature rabbits, a 2cm segment of the tibial diaphysis was isolated by making two transverse osteotomies. When the diaphyseal segment was created after death, with only its peristomal circulation uninterrupted, afferent vessels were observed, after arterial perfusion, to penetrate the cortex centripetally. With only its medullary circulation uninterrupted, afferent vessels were observed to penetrate the cortex centrifugally. As expected, when both circulations were interrupted no cortical vessels were demonstrated.

In addition, the diaphyseal segment was created in live animals and the peristomal, medullary (nutrient) and epiphyseal-metaphyseal circulations respectively were isolated so that the nutrition of the segment depended entirely on the isolated circulation. The animals were killed at intervals from 1 day to 4 weeks. Cortical vessels were perfused up to 2 weeks post-operatively but not afterwards in diaphyseal segments which were dependent on the medullary circulation. Whereas, cortical vessels were not perfused until 2 weeks and afterwards in diaphyseal segments that were dependent on the peristomal circulation. Cortical vessels were not perfused in the segments dependent on epiphyseal-metaphyseal blood supply during the period of study. All vascular isolation procedures were associated with cortical osteocyte loss.

It was concluded from these studies that:
1. The presence of periosteal tissue in close association with the fracture (osteotomy) may be necessary for normal healing;

2. An intact periosteal circulation may be necessary if an isolated diaphyseal segment (fracture fragment) is to continue to be adequately perfused; and, that

3. Cortical or medullary vessels perfused in fractures created after death or within days of fracture in live animals may not be perfused a few weeks later because they may sustain damage during the healing period due to post-injury (post-operative) oedema.

The segmental models employed in this study have not been previously used for vascular isolation and, on the basis of the perfusion studies, seem to show clearly the importance of the periosteal circulation. However, the fact that a vessel has been perfused does not prove conclusively that it is functional or vice versa. Many workers have expressed reservations about the accuracy of perfusion techniques in assessing the blood supply of bone. It has been suggested that they do not produce consistent results. Others suggest that the pressure required to inject substances such as barium sulphate may open up vessels that are not normally patent. Perfusion techniques were deliberately chosen for this study because it was proposed to demonstrate potential routes of supply. No other techniques are suitable for this. Every effort has been made to ensure perfusion at physiological pressures and the results were consistent within groups of animals studied. Nevertheless, further work is required to
confirm the findings from perfusion studies as reported in this thesis, for example, by combining these with microsphere or other techniques which measure blood flow.

The stability at the osteotomy sites in the operated tibiae in the recovery experiments (see sections 4.0 and 5.0) was probably different from one experimental model to another. A fracture is presumably more stable following intramedullary nailing compared with application of a sheath and plaster of Paris cast immobilisation. Motion at the fracture site is believed by some workers, notably Hicks (1963), to be a potent cause of delayed union. If this is true, then it may be assumed that the effect of motion on subsequent pathophysiological events was probably different from one experimental model used in this study to another. On the other hand, rigid immobilization affects both re-vascularisation and healing (Rhinelander 1972 & 1974, McKibbin 1978) and, therefore, would have unduely influenced the results. Nevertheless, it would be interesting to compare the results from plaster of Paris cast immobilised osteotomies with ones obtained using an external fixator.

Other findings suggest that osteocyte loss is not a good indicator of the state of cortical vascularity. For instance, normal looking osteocytes were still observed in the cortex 4 weeks after the application of a nail and sheath to the diaphyseal segment (see section 5.3) in which circumstances a blood supply could not conceivably have been present. Osteocyte loss did not follow any consistent or anatomical pattern thus suggesting that there is no direct relationship
between osteocyte loss and vascular territories. Thus, conclusions regarding blood supply of bone cannot be safely based on the presence or absence of osteocytes in the cortical lacunae.

The crux of the delayed union problem is the inability to bridge the fracture gap. The question then arises as to whether this is due to poor vascularity at the fracture site or to inadequate number of osteogenic cells due to severe periosteal damage. Or is there something at the fracture gap which inhibits healing? Some of the answers appear to be provided by the findings in this study.

Post mortem studies have not been previously used to obtain baseline data and, therefore, there has been a gap in our understanding of the pathophysiological (secondary) response of the osseous vasculature to fracture. Such studies have been reported in this thesis and a possible mechanism for soft tissue damage being related to delayed union is proposed. In each experimental model, particularly the nailed osteotomy model (see section 4.1) and nailed diaphyseal segment model (see section 5.1), cortical vessels demonstrated to be present in the post-mortem studies had disappeared at 1 day and at 1 week after operation but reappeared at 2 or at 4 weeks. This was presumed to be due to post-operative oedema. Therefore, it is suggested that, where the nutrient blood supply has been damaged, a potential route of supply exists, at least, from the periosteum, but that this can be temporarily interfered with by post-operative (post-injury) oedema. It is probable that the severity and duration of this post-injury oedema and,
thus, the duration of relative ischaemia of fracture fragments depend on the severity of the initial soft tissue damage.

According to this concept, high energy violence would lead to severe soft tissue damage which in turn would increase or prolong post-soft injury oedema. Consequently, the return of blood supply to the fracture fragments may be delayed or the blood supply may be diminished in the immediate post-fracture period. Many authors, notably Trueta (1963) have demonstrated a close relationship between osteogenesis and vascularity. Thus, diminished or delayed blood supply to fracture fragments, particularly in the crucial early proliferative phase, may cause the affected fracture to heal slowly or not at all. Perhaps active medical measures should be undertaken routinely to reduce tissue oedema in fractures in which it is suspected that severe concurrent soft tissue injury has been sustained. Perhaps serial measurements of serum CPK levels in the immediate post-fracture period could be used to monitor the response to therapy.

This study also suggets a potential role for severe damage to the periosteum in the development of delayed fracture healing. There are two possible mechanisms. Periosteal damage may lead to delayed union by virtue of a decrease in the total number of osteogenic stem cells initially available for proliferation. However, callus hypertrophy, in which the osteogenic potential of the tissues appears to have been overstimulated, may co-exist with non union (Judet et al. 1958, Brasher 1965, Weber & Chech 1976). Therefore, other factors related to severe periosteal damage
may prevent the fracture gap from being bridged with bone. For example, the periosteum may elaborate local humoral factors which stimulate cellular processes such as migration and differentiation. In the clinical study, the mean sequential serum osteocalcin levels after fracture suggested that osteoblast function was depressed but not abolished in delayed healing (see section 2.2). The latent powers of osteogenesis in slowly uniting fractures are also demonstrated by the fact that successful union is achieved by methods such as "shingling" (Dunn 1939) and "petalling" (Jarry & Uthoff 1960) which do not rely on a bone graft. Some workers have produced experimental evidence to suggest that the periosteum may elaborate humoral (?growth) factors which may affect bone growth (Wray & Schneider 1969, Crilly 1972). It is not inconceivable that severe periosteal damage suppresses the production of these growth factors and this concept deserves investigation.
7.0 GENERAL SUMMARY
1. Fractures of the adult tibial shaft often result in considerable morbidity because many are prone to slow healing. The reasons for this are not known and, therefore, prevention is not possible.

2. In this thesis, the problem has been re-examined, beginning with a clinical study (including radiological, biochemical and scintigraphic techniques) of 100 closed adult tibial shaft fractures treated by closed methods and then examining aspects which arose from this in the laboratory. Post-fracture soft tissue damage, fracture healing and blood supply were studied in the tibial diaphysis of mature rabbits using appropriate experimental models.

3. The true prevalence of delayed union was 19%. Of the fractures not united at this time, 15 were united at 30 weeks with continued conservative treatment. Only 4 fractures were operated upon because no further progress with regards to healing was anticipated by the treating surgeons. Of the clinical factors examined, only the severity of trauma appeared to be related to delayed union.

4. The serum CPK levels rose progressively for several hours after fracture but returned to normal after 3 days, and correlated closely with severity of trauma. The rise was also higher for patients developing delayed union compared to patients with normal union.

5. Serum levels of calcium and inorganic phosphate rose from
baseline values obtained within 24 hours of fracture to peak 2 weeks later and, thereafter, progressively declined during the healing period. The decline in values occurred more rapidly in delayed compared with normal union.

6. Serum levels of osteocalcin were generally lower in patients with delayed union and the differences in values compared with normal union was statistically significant at 16 weeks.

7. Serum somatomedin activity was not influenced by severity of trauma and was within normal limits in both normal and delayed union.

8. Sequential scintigraphic examination revealed that, of all recently published methods, only the A/C ratio of uptake over the fracture site relative to an adjacent site over the same tibia, with a cut off value of 2.0, clearly separated normal from delayed union.

9. Observations from experimental fractures of the mature rabbit tibial diaphysis revealed that different forces produce different fracture types and that these are associated with different patterns of soft tissue damage. Transverse fractures produced circumferential laceration of the periosteum and complete transection of the marrow but spiral fractures produced longitudinal periosteal laceration and incomplete marrow damage. For any given force, less was required to produce transverse than spiral fractures and, therefore, more remained to be expended in the soft tissues and produce
further damage. Although the nutrient artery may be interrupted by fractures, perfusion of the main trunks on either side of, and up to, the fracture line may still be observed due to filling via available collateral channels.

10. The relative contribution of the periosteal, medullary and intra-cortical tissues respectively to the healing of an experimental fracture of the mature rabbit tibial diaphysis was studied by selective tissue exclusion techniques. Only the presence of periosteal tissue in close association with the fracture (osteotomy) was found to be necessary for normal healing.

11. The relative contribution of the periosteal, nutrient and epiphysio-metaphysial circulations respectively to cortical blood supply was investigated by vascular isolation techniques using a diaphyseal segment model created in the mature rabbit tibia. Cortical arterial perfusion was observed in the segments before 2 weeks but not afterwards with the isolation of the nutrient circulation and at 2 weeks and afterwards with the isolation of the periosteal circulation. Cortical arterial perfusion was not observed with the isolation of the epiphysio-metaphysial circulation.

12. Cortical or medullary vessels perfused in fractures created after death or within days of fracture in live animals were found not to be perfused from a few hours to weeks later because they may sustain damage during the healing period due to post-injury (post-operative) oedema. This provides a possible mechanism by which severe soft tissue damage may lead
NAME AND INTENDED USE
IN-SOMC kit for radioimmunoassay of somatomedin C in human serum.

INTRODUCTION

Somatomedin C is a 70 amino acid peptide which is part of a family of growth factors which includes insulin-like growth factors I and II, somatomedins A and B, and IGF-1. Somatomedin C was recently found to be identical in structure to IGF-1 (16) whose sequence was published in 1978 by Hindmarsh and Haddad (22). There are similarities between protein A and somatomedin C that both contain A and B peptides with a C-peptide linker and both have internal disulfides. Somatomedin C is highly basic in nature due to its amino acid composition and circulates bound to high molecular weight proteins of approximately 140,000 daltons (6).

Somatomedin C mediates the growth promoting actions of growth hormone. It is released from a variety of tissues in the body in response to growth hormone and is under the control of a feedback mechanism forming a tightly coupled loop between the hypothalamus and the pituitary gland (24).

Other than growth hormone, two of the most important factors influencing somatomedin C concentration in the serum are age and sex. Somatomedin C levels are lowest at birth and rise throughout infancy and childhood. The highest levels are attained during adolescence and decline after adulthood. Females exhibit slightly higher somatomedin C levels than males during adolescence and adult years (1,26).

Somatomedin C measurement has been advocated as a screening and management tool in growth hormone deficient children (29). Its use in diagnosis along with growth hormone measurements (22) or as a tool to assess a child's response to administered growth hormone (13) has led it to a prominent place in the endocrine laboratory, particularly when dealing with growth disorders.

A second major use of somatomedin C measurement is in the diagnosis and treatment of acromegaly (21). Somatomedin C levels may be helpful to assess the results of bromocriptine treatment of acromegaly (23).

PRINCIPLE

The principle of the assay is based on competition between iodine 125 labelled somatomedin, and somatomedin C contained in standards or in specimen to be assayed, for a fixed and limited number of antibody binding sites. After the incubation period, the amount of labelled somatomedin bound to the antibody is inversely related to the amount of unlabelled somatomedin present in the sample.

The methodology proposed for separation is based on the use of an immunoprecipitating reagent which contains a second antibody and polyethylene glycol.

The main steps of the assay are:
1. Guanidine extraction for serum samples
2. Addition of labelled and rabbit anti-somatomedin C, followed by two hour incubation at 2-8°C.
3. Addition of iodine 125 somatomedin C and second incubation for 20 hours at 2-8°C.

4. Addition of immunoprecipitating reagent, and incubation for 2 hours at 2-8°C.

5. Centrifugation and counting.

3. DESCRIPTION

The expiration date printed on the kit label corresponds to that of the tracer. Each kit contains reagents sufficient for 65 tubes.

The reference is 145-SOMC

| Zero standard | 1 vial |
| Sonostimedin C standards | 5 vials |
| 125I sonostimedin | 1 vial |
| Control serum | 1 vial |
| Immunoprecipitating reagent | 5 vials |
| Columns | 65 |

These reagents stored at -15°C or lower upon receipt, are stable until the date indicated on the label of each vial.

3.1 Zero standard = lyophilized reagent

It contains RSA-borate buffer and sodium azide.

3.2 Sonostimedin C standards = lyophilized reagents

They contain human intact sonostimedin C at nominal concentrations of 4.5-95 nanomolar (0.1% of 3.8 units/mg) prediluted in RSA-borate buffer and sodium azide. Exact values are assigned with each lot.

3.3 Sonostimedin C antisera (rabbit) = lyophilized reagent

It contains rabbit anti-sonostimedin C serum diluted in RSA-borate buffer with heparin containing merthiolate.

3.4 125I sonostimedin C = lyophilized reagent

It contains human synthetic sonostimedin C (13-70) labelled with iodine-125, diluted in RSA-borate - EDTA buffer with sodium azide. The radioactivity is approximately 74 kBq (2 mCi) at the preparation date.

3.5 Control serum (level 1 and 2) = lyophilized reagents.

They contain human serum spiked with the appropriate amount of intact human sonostimedin C and sodium azide.

3.6 Immnoprecipitating reagent = lyophilized reagent

It contains normal rabbit serum pre-precipitated with goat anti-rabbit serum and polyethylene glycol (PEG), diluted in RSA-borate buffer with merthiolate.

3.7 Columns

Plastic columns contain octadecyl-silica (Sep-pak). Columns must be "washed" before applying sample. The ODS-silica columns may be stored at room temperature.

6. EQUIPMENT AND MATERIALS REQUIRED, BUT NOT SUPPLIED

- distilled water
- disposable borosilicate glass tubes (12x75 mm)
- temperature controlled centrifuge
- vortex type mixer
- pipetting devices
- micropipeters calibrated to deliver 50, 200 and 500 pl
- repeating dispensers, calibrated to deliver 200 and 500 pl.
- gamma counter suitable for measuring

Materials and reagents required for ODS Column Extraction

1. 20 ml disposable syringes
2. Isopropyl alcohol
3. Methanol, 100% grade or better
4. 6 N acetic acid
5. 0.5 N hydrochloric acid
6. Compressed air supply
7. 12°C, water bath
8. 16 x 100 mm glass tubes

5. SPECIMEN COLLECTION AND PREPARATION

Collection and Preparation of Serum

Collect blood by venipuncture in a 5 or 10 ml evacuated glass tube. Allow the blood to clot at room temperature. Centrifuge for 15 minutes at 1300 g to obtain hemoiacytolytic serum. No additives or preservatives are required to maintain the integrity of the sample. All plastic, glassware or other material coming into contact with the specimen should be entirely free of any contamination. EDTA or heparinized plasma may cause slightly lower results.

Storage of serum

The serum should be promptly separated from the cells assayed. For long term storage, specimens should be frozen at -20°C. Specimens can be frozen and thawed.
Extraction of Serum with ODS-Silica Columns

A. Pipette 250 µl of controls and unknown samples into a 12 x 75 mm glass test tube and add 1 ml of 0.5 N HCl. Vortex gently until well mixed.

B.1 Attach each ODS-silica column to a syringe hub.
B.2 Add 3 ml of reagent grade isopropanol to each syringe.
Press through the syringe using the syringe plunger.
Remove the ODS-silica column.′
Replace the ODS-silica column in the syringe.
B.3 Add 2 ml of HPLC grade methanol.
Press through the syringe.
Remove the ODS-silica column.′
Replace the ODS-silica column in the syringe.
B.4 Add 3 ml of 6% acetic acid.
Press through the syringe.
Remove the ODS-silica column.′
Replace the ODS-silica column in the syringe.
Repeat step B4.

C.1 Apply the sample from step A.
Push slowly through the cartridge at a flow rate of 1 ml per 2-3 minutes.
Remove the ODS-silica column.
Remove the plunger.
Replace the column in the syringe.
C.2 Wash the ODS-silica column two times with 10 ml of 6% acetic acid.

D. Collect the eluate in 16 x 100 mm glass tubes by eluting the peptide with 6 ml of HPLC grade methanol. Push slowly so that the methanol remains in contact with the ODS-silica column for a minimum of 3 minutes.

E. Evaporate the methanol to dryness in a 37°C water bath using a stream of air or nitrogen.

F. Reconstitute the dried samples with 500 µl of 0 standard and incubate at 37°C for 30 minutes to ensure complete reconstitution. A sample that is expected to be elevated should be reconstituted with 1.0 ml of 0 standard at this time. The final result should be corrected by the appropriate dilution factor (see calculation section).

G. Assay 50 µl of the serum extracts in duplicate in the radiommununoassay.

* Remove the ODS-silica column from the end of the syringe before removing the plunger to avoid contaminating the syringe contents with the fluid in the ODS-silica column.

4. ASSAY PROCEEDURE

6.1 Reconstitution of reagents

- Reconstitute the lyophilized reagents and allow any frozen reagents to thaw completely. Do not allow reagents to reach temperature above 20-25°C. Allow the reagents to stand for 15-20 minutes for complete reconstitution. Mix all reagents gently before using.

- Reconstitute the zero standard with 20 ml distilled water.
Store at -15°C or lower.

- Reconstitute the standards with 0.5 ml distilled water. Store at -15°C or lower.

- Reconstitute the antiserum with 14 ml distilled water. Store at -15°C or lower.

- Reconstitute 6.74 sodiumsodium C with 10 ml distilled water. Store at -15°C or lower.

- Reconstitute the immunoprecipitating reagent with 35 ml distilled water. Mix thoroughly until the suspension appears homogenous and then allow the slid to stand for a minimum of 30 minutes at room temperature with occasional mixing. Store at -15°C or lower.

- Reconstitute the control serum with 1 ml distilled water. After reconstitution, treat the control serum as a fresh serum. Store at -15°C or lower.

6.2 Preparation of tubes

The following tubes are used for the assay:

1 group for the total activity determination.
NO group for non-specific binding determination.
Zero group for 0 point and determination of binding ability.
Standard groups for the determination of the standard curve.
Check group for the control serum.
Sp group for unknown samples.

NB: It is recommended to perform the assay at least in duplicate.

6.3 Distribution of reagents and incubations

Bring the reagents to room temperature (18-25°C) prior to use.
Distribution of reagents to tubes is performed at room temperature.
After the distribution, mix the contents of each tube with a Vortex type mixer and incubate.
7. CALCULATION OF RESULTS

- For each group of tubes, compute the mean counts after subtracting the background.
- Evaluate the % binding ability of the system

\[
\% \text{ binding} = \frac{cpm \text{ Std } - cpm \text{ T}}{cpm \text{ T}} \times 100
\]

- The net counts of each standard and sample should be expressed as a percentage of the zero group

\[
\% \text{ binding} = \frac{cpm \text{ Std or Std } - cpm \text{ NSB}}{cpm \text{ Std } - cpm \text{ NSB}} \times 100
\]

Draw the standard curve by plotting the \% binding versus concentrations (mmol/l).

It is recommended to use semi-log or log-log coordinates.

Interpolate the levels of somatostatin C in the unknown samples from the plot.

Correct for appropriate reconstitution volume. For example:

- If 250 ml of serum was extracted and reconstituted with 500 ml of 0 standard, multiply the value interpolated from the curve by 2.
- If 250 ml of serum was extracted and reconstituted with 1.0 ml of 0 standard, multiply the value interpolated from the curve by 1.25.

Calculation example:

The following worksheet and standard curve example must be used as an illustration.

<table>
<thead>
<tr>
<th>Groups of tubes</th>
<th>Mean cpm (Std)</th>
<th>Mean cpm (NSB)</th>
<th>% binding x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>1565</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSB</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 Standard</td>
<td>6413</td>
<td>6012</td>
<td>100</td>
</tr>
<tr>
<td>Standard 4.5 mmol/l</td>
<td>4897</td>
<td>4916</td>
<td>76.7</td>
</tr>
<tr>
<td>Standard 6 mmol/l</td>
<td>4084</td>
<td>3733</td>
<td>61.4</td>
</tr>
<tr>
<td>Standard 12 mmol/l</td>
<td>2865</td>
<td>2516</td>
<td>43.3</td>
</tr>
<tr>
<td>Standard 32 mmol/l</td>
<td>1894</td>
<td>1561</td>
<td>25.8</td>
</tr>
<tr>
<td>Standard 48 mmol/l</td>
<td>96</td>
<td>581</td>
<td>4.6</td>
</tr>
</tbody>
</table>
9. SPECIFIC PERFORMANCE CHARACTERISTICS

9.1 Specificity
The antiserum used in the test shows cross-reactivity as follows:
- Rat MSA < 0.01
- R.D.H < 0.01

9.2 Sensitivity
It has been determined as being 2 mmol/l

9.3 Precision
Serum samples at different concentrations have been studied:
- within the same assay with n measurements
- between n assays with different lots of reagents.

### Within Assay

<table>
<thead>
<tr>
<th>Samples</th>
<th>% mmol/l</th>
<th>S.D. mmol/l</th>
<th>% Co.V</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>12.2</td>
<td>1.45</td>
<td>11.90</td>
<td>20</td>
</tr>
<tr>
<td>High</td>
<td>27.9</td>
<td>3.65</td>
<td>13.08</td>
<td>20</td>
</tr>
</tbody>
</table>

### Between Assay

<table>
<thead>
<tr>
<th>Samples</th>
<th>% mmol/l</th>
<th>S.D. mmol/l</th>
<th>% Co.V</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>11.7</td>
<td>1.89</td>
<td>16.31</td>
<td>20</td>
</tr>
<tr>
<td>High</td>
<td>17.9</td>
<td>2.99</td>
<td>16.70</td>
<td>7</td>
</tr>
</tbody>
</table>

9.4 Accuracy
The accuracy of the assay can be evaluated by the recovery and the dilution test.
The recovery of the assay was addressed by adding known amounts of standard to sample.

<table>
<thead>
<tr>
<th>Background</th>
<th>Standard Added mmol/l</th>
<th>Expected value mmol/l</th>
<th>Measured value mmol/l</th>
<th>Percent Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.9</td>
<td>5</td>
<td>18.9</td>
<td>17.3</td>
<td>93</td>
</tr>
<tr>
<td>13.9</td>
<td>10</td>
<td>23.9</td>
<td>23.0</td>
<td>106</td>
</tr>
<tr>
<td>13.9</td>
<td>20</td>
<td>33.9</td>
<td>33.1</td>
<td>100</td>
</tr>
<tr>
<td>No. 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29.2</td>
<td>5</td>
<td>29.2</td>
<td>29.3</td>
<td>87</td>
</tr>
<tr>
<td>29.2</td>
<td>10</td>
<td>36.2</td>
<td>36.7</td>
<td>84</td>
</tr>
<tr>
<td>29.2</td>
<td>20</td>
<td>46.2</td>
<td>46.1</td>
<td>73</td>
</tr>
<tr>
<td>No. 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.9</td>
<td>5</td>
<td>21.9</td>
<td>15.8</td>
<td>72</td>
</tr>
<tr>
<td>16.9</td>
<td>10</td>
<td>26.9</td>
<td>22.9</td>
<td>85</td>
</tr>
<tr>
<td>16.9</td>
<td>20</td>
<td>36.9</td>
<td>32.1</td>
<td>87</td>
</tr>
</tbody>
</table>
Appendix 2

MANUFACTURER USE

OCTOBER 1985

OSTE-PR is a radioimmunoassay kit manufactured by Coopagel ORIS
Industrie for a direct quantitative determination of osteocalcin (or BGP
Bone Gla Protein) in human serum.

1. INTRODUCTION

Osteocalcin or Bone Gla Protein (BGP) is a 49 Amino Acids protein (with
3'sCarboxy Glutamic Acid) and 5800 molecular weight. BGP is bone
specific and represents about 20% of non collagenous proteins.

BGP is synthesized in osteoblasts under the main influence of 1-25
Dihydroxy Vitamin D and once secreted binds strongly to hydroxyapatite.
It circulates in the blood and its level seems to reflect bone remodelling as it can be shown by the good correlation with
histomorphometric parameters of bone formation.

Although its biological function remains unknown, BGP is a specific
and sensitive marker of bone turnover and its assay may be useful in diagnosis and follow up of bone diseases such as post-menopausal
osteoporosis, renal osteodystrophy, hyperparathyroidism and
hyperparathyroidism-growth disorders, inflammatory chemation, myeloma,
Paget's disease.

2. PRINCIPLE

The principle of the assay is based on competition between iodine 125
labelled osteocalcin and osteocalcin contained in standards or samples
to be assayed for a fixed and limited number of antibody binding sites.

After the incubation period, the amount of labelled osteocalcin bound to
the antibody is inversely related to the amount of unlabelled
osteocalcin present in the sample.

The methodology proposed for the separation of bound and free fractions
is based on the use of an immunoprecipitating reagent in which a second
antibody is preprecipitated and in excess.

3. DESCRIPTION

The expiry date printed on the kit label corresponds to that of the
tubes.

Each kit contains sufficient reagents for 100 tubes:

- 1 STAND 1 to 5 vials
- anti-OSTEOCALCIN ANTISERUM 1 vial
- CONTROL SERUM 1 vial
- PR REAGENT 1 vial

These reagents stored at 2-8°C are stable until the expiry date
indicated on the label of each vial.

3.1 I-125 OSTEOCALCIN : lyophilized reagent

The labelled protein is a highly purified bovine osteocalcin. The reagent
contains human albumin in borate buffer, thimerosal and a red dye. The
radioactivity is approximately 74 kBq (2 mCi) at the preparation date.

3.2 OSTEOCALCIN STANDARDS : lyophilized reagents

The antigen used as standard is bovin osteocalcin -lyophilized in boreate
buffer containing human albumin. The exact amount is indicated on the
label of each vial. The values vary from one lot to the next but are
close to 0.5-1.1-3.3-10-30 ng/ml.

3.3 Anti OSTEOCALCIN ANTISERUM : lyophilized reagent

The antisera was obtained on rabbit by injection of bovin osteocalcin.
The reagent contains boreate buffer, human albumin, thimerosal and a blue
dye

Following the assay procedure, the antisera binds 50 to 60% of the amount of
labelled osteocalcin.

3.4 CONTROL SERUM : lyophilised reagent

It contains human serum and thimerosal.
The value varies from one lot to the next; the expected value is
indicated on the label of the vial.

3.5 PR REAGENT (Immunoprecipitating Reagent) ready to use reagent

It contains buffer, polyethylene glycol, sodium azide, an insoluble
coupled of sheep anti-rabbit gamma globulins and non immunised rabbit
gamma globulins.
4. EQUIPMENT AND MATERIAL REQUIRED BUT NOT SUPPLIED
- Distilled water
- Disposable plastic tubes
- Micropettes with disposable tips (100 µl, 200 µl, 1 ml)
- Vortex type mixer
- Multisample centrifuge (1500 g minimum) (refrigerated if possible)
- Tubes holders (preferably permitting the overturning of tubes)
- Y-counter suitable for measuring 125 I.

5. SPECIMEN COLLECTION
It is recommended to perform the assay on serum (haemolysed or hyperlipemic samples should not be used). The test specimen should be plasma using heparin (EDTA or citrate as anticoagulants have to be discarded). If the assay is performed within 2 hours, the samples should be kept 2-8°C. Otherwise they should be divided in aliquots and stored deep frozen (-20°C).

Dilutions
When high osteocalcin levels are suspected, the dilution is performed with the "O" standard contained in the kit.

6. ASSAY PROCEDURE
6.1 Reconstitution of reagents
- reconstitute the standards 1 2 3 4 5, the control serum with 0,5 ml of distilled water the standard "O" with 3 ml of distilled water a few minutes before use. Mix gently to homogenize.
- reconstitute the contents of the anti-OSTEOCALCIN antiserum with 10 ml of distilled water at the time of use. Mix gently to homogenize.
- reconstitute the contents of the tracer vial with 20 ml of distilled water at the time of use. Mix gently to homogenize.

6.2 Preparation of tubes
The following groups of tubes are required for the assay:
- T group for the determination of the total activity
- C group for the calculation of the non specific binding (NSB)
- O group for the determination of the binding ability.
- Standards groups for the determination of the standard curve
- Cx group for the calculation of NSB of the samples.
- Ex group for the samples to be assayed.

NB : It is recommended to perform the assay in triplicate for the T, C, 0, and standards groups and to use 1 tube for Cx and 2 tubes for Ex.

6.3 Distribution of reagents and incubation
The distribution of reagents is performed at room temperature (18-25°C);
Dispense in the following order
1 standards or samples
2 tracer
3 antiserum

<table>
<thead>
<tr>
<th>Groups of tubes</th>
<th>standards or samples</th>
<th>Tracer</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Std</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Cx</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Ex</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

All volumes are expressed in µl.

6.4 Addition of PR reagent
- keep the PR reagent until use at 2-8°C. Before distribution, the reagents must be hand shaken into a homogeneous suspension.
- distribute at room temperature 1 ml of PR reagent in all tubes (except those of T group).
- mix and incubate for 15 minutes at 2-8°C.

6.5 Centrifugation-Discarding of the supernatent-counting.
- centrifuge all the tubes (except those of T group) at 1500-2000 g for 15 minutes, if possible at 2-8°C.
- discard the supernatent by aspiration or decantation.
- the recommended method consists in overturning the tubes on an 'active',
  sink or a container suitable for collecting radioactive solutions.
  Shake the tubes and leave them overturned 10 minutes on absorbing
  paper.

N.B: All the tubes within the same assay must be treated identically;

Counting: Measure the radioactivity in each tube with a counter
suitable for measuring 1/1.

7. CALCULATION OF RESULTS

For each group of tubes, compute the mean counts after subtracting the
background. For the groups 5, 6, Std 1 to Std 5, the non specific C
activity will be subtracted whereas for the Sx groups, the corresponding
non specific Cx activity will be subtracted;

Evaluate the binding ability of the system by dividing the mean value of
the 'O group' by the mean value of 'T group';

\[
\frac{G - C}{B / T} = \frac{S - C}{S x - C x} \times 100
\]

The binding ability is comprised between 50 and 60%.

The mean counts for standards and samples is expressed as a percentage
of the 'O group'.

For the standards (B / B0) \( \% = \frac{S - C}{S x - C x} \times 100 \)

For the samples (B / B0) \( \% = \frac{G - C}{S x - C x} \times 100 \)

Calculation example

<table>
<thead>
<tr>
<th>Groups of Tubes</th>
<th>Mean cpm</th>
<th>B/T</th>
<th>B/B0 x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>23423</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1030</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14088</td>
<td>59.16</td>
<td></td>
</tr>
<tr>
<td>Std 1</td>
<td>12831</td>
<td></td>
<td>83.2</td>
</tr>
<tr>
<td>Std 2</td>
<td>11116</td>
<td></td>
<td>72.0</td>
</tr>
<tr>
<td>Std 3</td>
<td>7042</td>
<td></td>
<td>43.4</td>
</tr>
<tr>
<td>Std 4</td>
<td>3780</td>
<td></td>
<td>19.0</td>
</tr>
<tr>
<td>Std 5</td>
<td>2257</td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>Sx</td>
<td>8501</td>
<td>56.5</td>
<td></td>
</tr>
</tbody>
</table>

Warning: the values given in this table and the calculation example must
only be considered as illustrations.

- Draw the standard curve on semi-log or linear graph paper by plotting
  the standards B / B0 versus their concentrations.

- Read the sample values directly from the curve (Fig 2) and correct the
  read value with the dilution factor if needed.

8. EXPECTED VALUES

It is recommended for each laboratory to establish its own range of
normal values. As an example, the mean value obtained on 60 presumed
normal serum subjects is 5.3 ng/mL with 1.65 ng/mL as standard
deviation.

9. SPECIFIC PERFORMANCE CHARACTERISTICS

Sensitivity:

It has been determined as being 0.15 ng/mL
BIBLIOGRAPHY

A


Albinus B.S. in Academicarum Liber III., Verbeek, Leidae (1756)


Austin R.T. "Fractures of the tibial shaft: is medical audit possible?" Injury 9, 93 - 101 (1977)

B

Bach A.W. & Hansen S.T. "Delayed union, non union and malunion of the tibial shaft." in Surgery of the musculoskeletal system


Brookes M. in The blood supply of bone, Butterworths, London (1971)


Campbell W.C. in Operative orthopaedics, Saunders Co, Philadelphia (1939)

Carpenter E.B., Dobbie J.J. & Siewers C.F. "Fractures of the shaft of the tibia and fibula. Comparative end-results from various types of treatment in a teaching hospital." Arch. Surg. 64, 443 - 456 (1952)


Chiewitz O. & Hevesy G. "Radioactive indicators in the study of
phosphorus metabolism in rats." Nature 136, 754 (1935)


Crock H.V. in The blood supply of the lower limb bones in man, Livingstone, Edinburgh (1967)


de Marneffe R. in Reserches morphologiques et experimentales sur la vascularisation osseuse, Acta medica Belgica, Brussels (1951)


Duhamel H.L. "Sur une racine qui a la faculte de tiendre en rouge les os des animaux vivants" Mem. Acad. Roy. Sci. 52, 1 (1739)


Du Nouy P.L. in Biological time, Methuen, London (1936)


F


Ficat P. & Arlet J. in Ischaemia and necrosis of bone (adapted by Hungerford D.S.), Williams & Wilkins, Baltimore (1980)


Fischer A. in Biology of tissue cells, Cambridge Univ. Press (1946)


Green N., French S., Johnson D. & Fingerhut A. "Roentgenologic findings in non union of long bone fractures." Invest. radiol. 2, 17 - 220 (1971)

Gregg P.J., Barsoum H.K. & Clayton C.B. "Scintigraphic appearance of the tibia in the early stages following fractures." Clin. Orthop. 175, 139 - 146 (1968)


Gregg P.J., Clayton C.B., Fenwick J.D., Ions G.K., Miller S.H.R. & Smith S.R. "Static and sequential dynamic scintigraphy
of the tibia following fracture." Injury 17, 95 - 103 (1986)
H
Haller A. von in Experimentorum de ossium formatione, Francisci Grasset, Lousanne (1763)
Havers C. in Osteologia nova, or some new observations of the bones etc., Samuel Smith, London (1691)


Henderson H.S. "Ununited fractures." J. Amer. Med. Assos. 86, 81 - 86 (1926)


Hicks J.H. "Non-union of fractures." Lancet 1, 86 - 88 (1963)


Hunter W. "Of the structure and diseases of articulating cartilages." Phil. Trans. R. Soc. 42, 514 - 521 (1743)

Illingworth G.I. & Schiess P.A. "Strontium 87m in the prognosis

J


K


Kaski P. "Osteomedullography of the tibia. Intraosseous phlebography with compression of the soft tissue veins." Acta Radiol. suppl. 312 (1971)


Koekenberg L.J.T. in Vascularisation in the healing of fractures, Born NVU, Assen/Amsterdam (1963)


Kunse (1933) as cited by Brookes M. in The blood supply of bone, Butterworth, London (1971)

Kuntscher G. in The callus problem, Green, St. Louis (1968)

L


Lamas A., Amado D. & da Costa J.C. "La circulation du sang dans los." Presse med. 54, 862 - 863 (1944)


Leriche, R. & Policard, A. in The normal and pathological physiology of bone, Mosby Co., St. Louis (1928)

Lexer, E., Kuliga, P. & Turk, W. in Untersuchungen über knochenarterien, Hirschwald, Berlin (1904)

Lexer, E. "Über die entstehung von pseudarthrosen nach frakturen und nach knochentransplantationen." Arch. klin. Chir. 119, 520–607 (1922)


Macewen, W. in The growth of bone: observations on osteogenesis, Maclehose, Glasgow (1912)


Matin, P. "The appearance of bone scans following fractures including immediate and long term studies." J. Nucl. Med. 20, 1227–1231 (1979)

McAuley, G.O. "The blood supply of the rabbit femur in relation to repair of cortical defects." J. Anat. 92, 605 (1958)

McCarthy, I.D. & Hughes, S.P.F. "Extraction of 99m Tc-methylene
diphosphonate as a function of bone blood flow."

McKibbin B. "The biology of fracture healing of long bones." J. Bone
Circulation, Arlet J., Ficat R.P. & Hungerford D.S. eds.)
Williams & Wilkinson, Baltimore (1984)

McLone B. "The biology of fracture healing of long bones." J.

McLean F.C. & Urist M.R. "End-result observations influencing

Assos. 159, 1088 - 1093 (1955)

McLean F.C. & Urist M.R. in Bone: an introduction to the

physiology of skeletal tissue, 2nd ed., Univ. Chicago Press,
Chicago (1961)

McPherson A. & Shaw N.E. "New concept of capillary circulation
in bone." Lancet 1, 1285 -1286 (1961)

Misol S., Samaan W. & Ponseti I.V. "Growth hormone in delayed

Morgan J.D. "Blood supply of growing rabbit's tibia." J. Bone
Joint Surg. 41B, 185 - 203 (1959)

Morton D.B. & Griffiths P.H.M. "Guidelines for the recognition
of pain, distress and discomfort in experimental animals and an


Muheim G. "Assessment of fracture healing in man by serial
87strontium-scintimetry." Acta Orthop. Scand. 44, 621 - 627
(1973)

Mulholland M.C. & Pritchard J.J. "The fracture gap." J. Anat
93, 590 (1969)

N

University (1981)

42A, 625 - 636 (1960)

Nicoll E.A. "Fractures of the tibial shaft. A survey of 705
cases." J. Bone Joint Surg. 46B, 373 - 397 (1964)

Nordstrom H., Lenquist S., Lindell B. & Sjoberg H.C.
"Hypophosphatæmia in severe burns." Acta chir. Scand. 143, 395
- 399 (1977)

Norusis H.J. in Introductory statistics guide: SPSS,

O

Oestern H.-J. & Tscherne H. "Pathophysiology and classification
of soft tissue injuries" in Fractures with soft tissue injuries


Perkins G. in Fractures and dislocations, Athlone Press, London (1958)


Phemister D.B. "Repair of bone in the presence of aseptic necrosis resulting from fractures, transplantations and vascular obstructions." J. Bone Joint Surg. 12, 769 - 787 (1930)


Pritchard J.J. in Recent advances in anatomy (Golby F. & Harrison R.J.) Livingstone, Edinburgh (1961)


Rhinelander F.W. "The circulation of bone" in The biochemistry and physiology of bone (Bourne G.H.), Acad. Press, N.Y. (1972)


Robinson R.A. in Significance of phosphoric esters, New York (1932)

Rudd G.V. "The calcium and phosphorus content of the blood in fractures." Med. J. Aust. 11, 399 - 401 (1927)

Sakellarides H.T., Freeman P.A.J. & Grant B.D. "Delayed union and non union of tibial shaft fractures." J. Bone Joint Surg. 46A, 557 - 569 (1964)


Sinclair N. in Fractures, Constable Co., London (1931)


Smith R. in Biochemical disorders of the skeleton, Butterworths, London (1979)


Testut L. & Latarjet A. W. "Traite d’anatomie humaine, Doin, Paris (1948)


Trueta J. & Cavadas A.X. "Vascular changes caused by the


Trueta J. in Studies of the development and decay of the human frame, Heinemann, London (1968)


Urist M.R. "Bone formation by osteoinduction." Science 150, 893 (1965)

Urist M.R. "Growth hormone and skeletal tissue metabolism" in The biochemistry and physiology of bone (Bourne G.H.), Acad. Press, N.Y. (1972)

V


W


Watson Jones R. in Fractures and joint injuries, Livingstone, Edinburgh (1943)


Weiland A.J., Berggren A. & Jones L. "The acute effects of


Y


