THE PREVENTION OF INFECTION IN OPEN FRACTURES

AN EXPERIMENTAL STUDY OF THE EFFECTS OF FRACTURE STABILITY AND OF ANTIBIOTIC THERAPY

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ABSTRACT

An experimental model of a contaminated open fracture has been developed in rabbits, using a reproducible midshaft fracture of the tibia. This model has been used to:

1) Test the hypothesis that stable fixation of an open fracture will reduce its susceptibility to infection.

2) Assess the effect of antibiotics on infection rate, with particular reference to the delay in administering the initial dose.

The pattern of fracture healing was initially determined for stable and unstable fixation, without inoculation with bacteria. Fractures fixed with a dynamic compression plate ("stable" group) healed by primary bone union, while fractures stabilised with a loose-fitting intramedullary rod ("unstable" group) healed by external callus formation.

Forty-one rabbits were used in the definitive study of the effect of stability. All fractures were inoculated with Staphylococcus aureus in a standard concentration. There were twenty rabbits in the stable group (compression plate) and osteomyelitis developed in seven (35%).

Of the twenty-one rabbits in the unstable group (loose-fitting intramedullary rod), fifteen (71%) became infected. This difference in infection rate is statistically significant ($p<0.02$).

The "rod-fixed fracture" model had the highest infection rate and was therefore used to study the effect of antibiotics. Fifty-one rabbits were used; a single intramuscular injection of cephradine was given to each animal at varying times in relation to inoculation with bacteria. Although the maximal reduction in infection rate was observed when the antibiotic was given before inoculation with bacteria, a 40% decrease in the infection rate was still seen when the antibiotic was given after bacterial inoculation. This effect persisted even if the initial dose of antibiotic was delayed four hours after inoculation.

These findings support the concept of stabilisation of open fractures in man; and suggest that appropriate systemic antibiotics should be routinely used in the management of open fractures in man, even if the treatment is delayed up to four hours after injury.

CANDIDATE'S DECLARATION

I declare that I am solely responsible for the planning and the conduct of the work contained in this thesis. The technical advice and assistance that I received are acknowledged overleaf. The interpretation of the results and the conclusions drawn are my own. Dr Leonard Harvey MRCPath (Senior Lecturer in Histopathology, University of Nottingham) assisted in the histological assessment and the independent radiological assessments were carried out by Dr Rod Mawhinney FRCR (Lecturer in Diagnostic Radiology, University of Nottingham).

All the work was carried out during my present appointment as Lecturer in Orthopaedic and Accident Surgery, University of Nottingham.

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ABBREVIATIONS USED IN THE TEXT

Statistical analysis

p	Significance value
NS	Not significant (p>0.05)
SD	Standard deviation
SEM	Standard error of the mean
x^2	Chi-square test
t	Student's "t" test
DF	Degrees of freedom

Other abbreviations

%	percentage
mm	millimetre
cm	centimetre
mg	milligram
kg	kilogram
ml	millilitre
mg/l	milligrams per litre
mA	milliamps
kv	kilovolts
Nm	Newton metres

rpm	revolutions per minute
CFU	Colony forming units
MIC	Minimum inhibitory concentration
MBC	Minimum bacteriocidal concentration
EDTA	Ethylenediaminetetra-acetic acid
AO	Arbeitsgemeinschaft fur Osteosynthesefragen (Association for the Study of Internal Fixation)
H & E	Haematoxylin and Eosin

CHAPTER 1

INTRODUCTION

Open fractures present a major problem to the orthopaedic surgeon. This is primarily because of infection, which is the main cause of amputation, non-union and poor functional and cosmetic results in these patients.

The management of contaminated wounds has been a major problem to surgeons through the ages. The descriptions of wound treatment in the Edwin Smith Surgical Papyrus, which dates from 1600-1500 BC, indicates that Egyptian physicians were dressing wounds with grease, honey and lint. In biblical times other methods of antisepsis were used : "and bound his wounds, pouring in oil and wine" (Luke 10:33,34).

There is little written evidence remaining about the practices of medieval surgeons. However, a 14th century French surgeon, Guy de Chauliac, advocated the enlargement of the wound to provide drainage, the removal of foreign bodies and the use of brandy in the dressings (Nicaise, 1890). An English surgeon, John Arderne of Newark, also practised in the same era and wrote of the necessity of keeping wounds clean so that they could heal without suppuration. He also advised that wounds should be dressed infrequently and allowed to heal from the bottom upwards (Mee 1938).

Yet the teachings of these men remained

largely unknown. Ambroise Pare (1510-1590) recorded that gunshot wounds were usually treated by cautery with boiling oil. Based on his experience as a military surgeon, Pare recommended enlarging the wound and removing all foreign material, before applying a salve to the wound.

Pare records graphically an injury he suffered when kicked in the shin by a horse. This caused an open fracture of his tibia "four fingers above the ankle". Pare instructed a colleague to enlarge the wound, remove a piece of devitalised bone and drain the haematoma. The leg was splinted and the wound left open to drain (see Figure 1.1). Although the wound discharged for some time, both soft tissue and fracture healed, but it was four months before he could walk without support.

During the first half of the 19th century fractures with open wounds were one of the most fatal forms of injury (Godlee 1917), and immediate amputation was often advised for such injuries. Although Lister described the use of carbolic acid as an antiseptic in the treatment of open fractures in 1867, credit must be given to Auguste Nelaton (1807-1873) who had been using alcohol dressings since 1852 (Atlee 1855). Hugh Owen Thomas (1834-1891) tried Lister's techniques, but found them no improvement on his own method of laying open the wound and washing it out with salt

The figure of a Leg fractured with a Wound, and bound up.

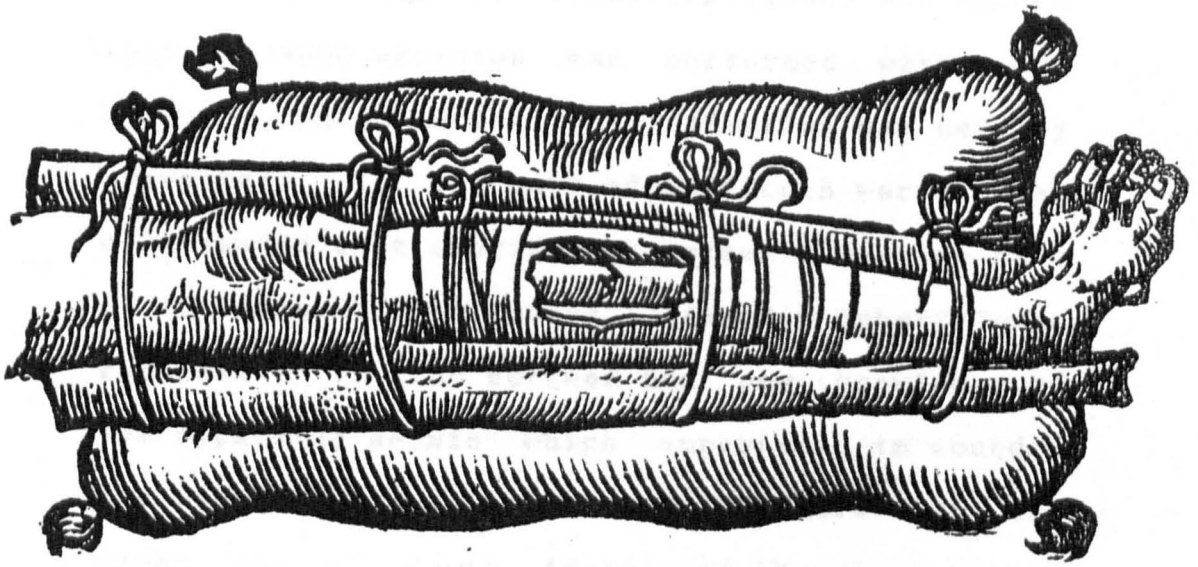


FIGURE 1.1 : Splintage of an open fracture of the tibia.

Reproduced from Pare, 1691.

and water (Keith,1919). It thus took some 20 years for Lister's method's to become widely practised, but then the antiseptic school reigned supreme until the Great War.

This delay occurred despite the experimental work of Friedrich (1898) who showed that if wound excision was performed within 4-6 hours of contamination, then infection was usually prevented. It was only after trench warfare was established that debridement became a routine part of the treatment of wounds. As Sir Robert Jones recorded : ".....no surgeon had any knowledge of the appalling sepsis which supervened in wounded men who had lain in the mud of Flanders. The dry clean sun of South Africa and the clean bullet wound had given us all a false impression of the nature of military surgery" (Watson, 1934). Following this experience, the thorough debridement of wounds (thereby removing necrotic tissue and foreign bodies) and the use of hypochlorite irrigation solution became mandatory.

At the start of the Great War, the mortality after open fracture of the femur approached 80%. It was Robert Jones who advocated the use of the Thomas splint to stabilise the fracture, and the mortality fell to 20% (Osmond-Clarke 1950). This concept of splintage was taken further by Trueta (1939) after his experiences in the Spanish Civil War. Based on

the work of Winnett Orr (1927) he practised extensive debridement of the wound, excising all the necrotic or doubtful tissue, and left the wound open. The limb was then immobilised in plaster which included the joint above and below; this plaster was retained as long as possible. Of 1073 open fractures treated in this way there were only six deaths. The osteitis rate was 7.6%, and a good functional result was seen in over 90% of patients.

During the Second World War the principles of treatment of adequate wound excision and splintage with Thomas splint or plaster cast were firmly established. It was possible to evacuate casualties rapidly to field hospitals where definitive surgery could be performed. The debate over whether closure should be primary or delayed was settled in favour of the latter. In a series of 5,042 consecutive open fractures treated in Europe, Cleveland and Grove (1945) reported spontaneous healing of the open wound in 42%, and that delayed primary closure was usually effective in the remainder. Using these methods they found that 87% of wounds were healed within ten weeks, and that the osteitis rate was 5.4%.

This policy of rapid evacuation, wound excision and delayed closure was adopted in both the Korean and Vietnam conflicts. In the latter Heaton et al (1966) reported infection rates as

low as 2.6% after open fractures. Although most historical data come from military surgeons, the lessons learnt have been translated into civilian practice. The principles of early and thorough wound excision, copious wound irrigation and delayed closure are now widely accepted. However, despite these measures infection rates of between 2.4% and 13.9% have been reported after open fractures in civilian practice (Gustilo and Anderson 1976, Patzakis et al 1974).

It is of more significance to study infection rates after different types of open fracture. Gustilo and Anderson (1976) classified open fractures into three types:

Type I: An open fracture with a wound less than one centimetre long.

Type II : An open fracture with a wound more than one centimetre long, without extensive soft tissue damage, flaps or avulsions.

Type III : Either an open segmental fracture or an open fracture with extensive soft tissue damage or a traumatic amputation.

There were no infections after Type I injuries in their series, but the infection rate in Type II injuries was 1.1% and in Type III injuries it was 8.9%. Other workers have reported similar patterns, but with higher infection rates in each of the three groups (Bauer et al 1962, Veliskakis 1959). The importance of preventing

such infection can be seen from the work of Hicks (1964), who reported that two-thirds of amputations after lower leg injuries were as a result of infection.

The prevention of infection is also important if reliable bone union is to occur. It is therefore necessary to understand the biology of fracture healing, and to know how the blood supply of bone is related both to fracture healing and potential infection.

Fracture Healing

The key step in fracture union is the formation of a bony bridge between the fragments. There are three mechanisms by which this can occur.

Healing by external callus.

If the periosteum remains intact then there is effectively a soft tissue tube linking the bone ends. Ham (1969) describes osteogenic activity sub-periosteally which results in the formation of two collars of bone which advance until they meet and establish a bony bridge. This primary callus response has been noted by McKibbin (1978) who observed in experimental studies that if the callus collars had not made contact within two weeks, they underwent involution. McKibbin describes this as a characteristic response, seen

in many differing circumstances and limited in duration. Providing the periosteal tube remains intact and the fracture gap is not large, then the primary callus response may be sufficient to produce bone union.

However, it is likely that other factors are involved in the healing of most fractures. The work of Trueta (1968) and Freidenstein (1968) suggests that osteogenic cells may arise from other sites, namely the vascular endothelium and distant bone marrow. It is not completely clear how these other factors are involved in inducing bone formation when the primary callus response is inadequate in producing union.

The presence of a distal fragment is important; it is well known that, after amputation, a primary callus response may occur, but normal callus is not seen. We also know from clinical experience of fracture healing that the amount of callus formed is in some way related to movement at the fracture site. It has been shown experimentally that callus formation is abolished in conditions of rigid internal fixation (Anderson 1965, Schenk 1978).

There is some evidence to suggest that bioelectrical potentials may be involved in osteogenic induction. Moreover, it is clear that electo-negativity favours bone formation (Bassett et al 1974). Whether these electro-negative

potentials arise from the fractured bone-ends as suggested by some workers (Friedenburg and Brighton 1966), or from the surrounding injured tissues as suggested by Lokietek et al (1974), is unresolved.

The search for a hormone which, when liberated from a fracture, would induce osteogenesis has been pursued since the 1920's. However, no substance has yet been identified. Despite this lack of success, the work of Goldhaber (1961) suggests that such an agent may exist, as he was able to induce heterotopic bone formation across a millipore filter. This work has been confirmed by Heiple et al (1968).

Although the precise way in which this external callus forms is not defined, it is clear that after the initial primary callus response, there is a second phase of bridging callus due to osteogenic cells derived from the surrounding tissues.

Medullary callus

The presence of callus within the medullary canal after fractures is well recognised. It is likely that this medullary callus is influenced by different factors. Mechanical stability has an inhibitory effect on external callus, while medullary callus is often seen in stable conditions (Olerud and

Danckwardt-Lilliestrom 1971, Rhinelander 1974). It seems to be largely derived from the fractured bone ends: for if the latter are cauterized experimentally callus formation is decreased (Templeton 1960). Medullary callus appears to be a late phenomenon in fracture healing and may continue for many months (McKibbin 1978).

Primary bone healing

It has been observed clinically that direct bone bridging of the fracture gap occurs under conditions of anatomical reduction and stable fixation (Danis 1949). This direct bone bridging has been confirmed histologically in dogs (Schenk and Willenegger 1967, Olerud and Danckwardt-Lilliestrom 1968), rabbits (Rahn et al 1971, Ashurst et al 1982) and in man (Schenk 1978). Under stable conditions and where the bone ends are in direct contact, healing occurs by Haversian re-modelling as osteons cross the fracture site. Inevitably there are areas where small gaps result. Providing these gaps are less than 300 microns wide, new bone is laid down in the fracture gap; there is then longitudinal reconstruction of the fracture site by Haversian re-modelling. Primary bone union requires conditions of stability; even a small amount of movement at the fracture site will inhibit the process.

Blood supply of bone

In normal resting bone there are three components of the afferent vascular system :

1. The principal nutrient artery, which crosses the diaphyseal cortex to enter the medullary cavity. This sub-divides into ascending and descending arteries, which sub-divide into arteries supplying the entire endosteal surface.
2. Multiple metaphyseal arteries, supplying the metaphyses. Terminal branches enter the medullary cavity and anastomose with terminal branches of the ascending and descending medullary arteries. These anastomoses will maintain the medullary circulation if the nutrient artery is damaged.
3. Periosteal arterioles, mainly derived from fascial and ligamentous attachments. These anastomose with terminal branches of the medullary arteries within cortical bone.

Experimental work in the rabbit (Gothman 1960a) and the dog (Rhinelander 1974) suggests that, in normal bone, the inner two-thirds of the cortex is supplied by the medullary arteries, while the outer third is supplied by the periosteal arterioles. Gothman (1960a) reported that in the rabbit there were numerous anastomoses between medullary and periosteal systems which penetrated throughout the cortex. In normal bone,

blood flow is centrifugal from medulla to periosteum (Rhinelander 1968, Brookes 1971). There is great capacity for increasing the vascularity of normal bone and Rhinelander (1968) has shown that, in the canine ulna, there is physiological stimulation of the resting medullary blood supply if the ulna of the opposite limb is fractured.

When a bone is fractured, all three components of the vascular supply (as previously described) are capable of enhancement, but in cast treatment of closed fractures it is the medullary system which predominates (Rhinelander and Baragry 1962, Rhinelander et al 1968). In undisplaced fractures there was immediate opening up of existing vascular channels; and it was the enhancement of the medullary circulation which dominated the healing process (Rhinelander and Baragry 1962). In contrast, the periosteal arterioles were the main <u>initial</u> source of blood supply in displaced fractures (Rhinelander et al 1968). However, the medullary supply was augmented via the anastomoses with the metaphyseal arteries and there was a gradual proliferation of the medullary arterioles. Under stable conditions medullary arterioles were seen to cross the fracture site within three weeks; later, the medullary circulation was responsible for the major part of the blood supply.

There is a fourth way in which a healing fracture receives a blood supply. This is the extra-osseus blood supply arising initially from torn blood vessels around the fracture site; it may become very extensive, derived from blood vessels in soft tissues some distance away (Gothman 1960c). This extra-osseus blood supply persists until replaced by the increasing vascularity of the medullary system. If the regeneration of this medullary system is delayed, the extra-osseus system persists (Rhinelander 1974).

Different methods of internal fixation will affect the blood supply of the bone and the fracture site in different ways. The application of a plate to a bone results in greatly reduced cortical vascularity beneath the plate (Olerud and Danckwardt - Lilliestrom 1968, Rhinelander 1974). This effect persists for up to six weeks. Whereas Olerud and Danckwardt-Lilliestrom (1968) reported that only the outer half of the cortex was affected, Rhinelander (1974) observed this avascularity in the full thickness of bone underlying the plate. He suggested that a plate blocks the efferent vessels in the underlying periosteum, and this stops the normal centrifugal flow of blood from medulla to periosteum.

The stabilising effect of a plate is beneficial to revascularisation at the fracture

site. Rhinelander et al (1967) observed blood vessels crossing an osteotomy one week later when the osteotomy was stabilised by a plate. This has been confirmed by Ganz et al (1970) in experimental studies in rabbits. They concluded that provided rigid internal fixation is maintained, the medullary circulation has a rapid rate of proliferation and regeneration, and that continuity of the medullary artery is restored in only a few days.

The insertion of an intramedullary nail will obviously affect the medullary circulation. Trueta and Cavadias (1955) studied the effect of intra-medullary nailing in experimental fractures of the tibia in the rabbit. They found, almost inevitably, that there was destruction of the nutrient artery, with signs of avascular necrosis in the inner two-thirds of the cortex. However, despite these ischaemic inner areas, there was a generalised increase in cortical vascularity, and there was evidence of medullary blood flow despite destruction of the nutrient artery. They suggested that if the medullary blood supply was interrupted, the periosteal vessels could assume the role of cortical blood supply.

Gothman (1960b) studied the effect of intramedullary nailing on the intact rabbit tibia. He reported that only exceptionally was there destruction of the medullary arteries; usually

one or more of the large branches of the medullary artery was present. There was a lively vascular response from the periosteal vessels. There was no histological evidence of avascular necrosis or bone infarction, suggesting that this vascular response of the periosteum was capable of maintaining cortical vascularity in the presence of either total or partial destruction of the medullary arteries.

In a further study, Gothman (1960c) examined the effect of intramedullary nailing on an experimental osteotomy of the tibia in the rabbit. He reported that there was vascular proliferation of the periosteal vessels and that there was a marked vascular response from the surrounding soft tissues. These vessels were derived from arteries some distance away. Remnants of the nutrient artery and associated cortical branches were also present. However, these persisting medullary vessels did not have as marked a proliferation as the periosteal vessels and the extra-osseus supply.

Rhinelander (1974) has reported that a loose-fitting intramedullary rod causes less damage to the medullary vasculature than a tight-fitting nail. Cortical bone was only de-vascularised where the rod was in contact with the endosteal surface; there was rapid regeneration of the medullary vascular system

where the rod was not in contact. Cross-sectional micro-angiograms showed the excellent vascular supply to cortical bone surrounding a loose-fitting rod (Rhinelander et al 1967).

If the medullary cavity is reamed before inserting a tight-fitting rod, considerable vascular damage occurs. The damage can be diminished by reducing the intramedullary pressure by means of a suction catheter inserted into the distal metaphysis (Danckwardt-Lilliestrom et al 1970). Using this method it was found that the percentage of avascular cortex was reduced from 20% to 7%. This work supports the view that the periosteal vessels assume the role of supplying the cortex if the medullary vessels are damaged.

All these reports were micro-angiographic studies following dye injection and are therefore static studies. There is only one comparative dynamic study of fracture site blood flow, namely that of Rand et al (1981). They compared the effects of compression plating and intramedullary nailing on the vascular supply to a standard fracture site. A transverse osteotomy was performed, on both tibiae in dogs. One limb was stabilised with a dynamic compression plate and the other with a fluted intra-medullary rod, after reaming of the intramedullary cavity with pressure reduction as already described. Medullary vascular regeneration was quicker and more marked

with a fluted rod than with a conventional cloverleaf nail (Rhinelander 1974).

Dynamic studies of blood flow in both the whole bone and at the fracture site were performed using a 85Sr clearance technique at varying times after operation. In both the compression plate group and the intramedullary nail group the whole bone blood flow reached a maximum after 14 days, with significantly higher levels after intramedullary nailing. The fracture site blood flow initiallly fell to 30% of normal in both groups, but rose to a maximum by 14 days. There was no significant difference in maximal blood flow at the fracture site between the two methods of fixation. However, blood flow remained at its peak for a significantly longer period of time in those fractures treated by intramedullary nailing. Although the compensatory mechanism is not defined, it is clear that if either the periosteal or medullary vascular systems are damaged, then the intact system can increase activity to compensate and allow union to proceed.

From the point of view of the practising orthopaedic surgeon, it is clear that he must endeavour to prevent further damage to any part of the blood supply to a bone. If an intramedullary nail is used, great care must be taken to avoid further damage to the periosteal system. If a plate is used, then regeneration of the medullary

circulation is achieved most rapidly in the presence of rigid fixation. If union is to be achieved and infection prevented then all steps must be taken to prevent the occurrence of tissue necrosis, restore the microcirculation and prevent contamination by bacteria.

Prevention of Infection

In recent years surgeons have investigated the use of two more methods to prevent infection in open fractures.

1. A more detailed understanding of vascular regeneration around a fracture has led to the recommendation that internal/external fixation be used to stabilise the fracture.
2. The development of powerful antibiotics has led to hopes that that these might be used to destroy any bacteria which have inoculated the wound.

Effect of stability

It has been stated that stabilisation of a fracture is the best prophylaxis against sepsis (Muller et al 1979). This argument has been further developed by Allgower and Border (1983) who advocate primary internal fixation in open fractures. They state firmly that "it has been the vast experience of the last decade that stably

fixed bone defends itself best against infection". Chapman (1982) felt that stabilisation of the fracture improved the tissue microperfusion and thus brought immune mechanisms (both cellular and humoral) into contact with bacteria. Stabilisation of the fracture also reduced both dead space and exudate formation. While admitting that this theory was based on some assumptions, Chapman asserted that recent animal experiments and clinical experience were in its favour.

What, therefore, is the "vast experience" quoted by Allgower and Border (1983) and the "recent .....clinical experience" suggested by Chapman (1982)? It is disturbing to find a lack of hard clinical data to support these confident claims. Rittmann et al (1979) treated 214 consecutive open fractures by primary internal fixation, and recorded an overall infection rate of 7%. Chapman and Mahoney (1979) reported a series of 101 open fractures treated by primary internal fixation with an infection rate of 10.6%. In the series of LaDuca et al (1980) there were 50 open fractures treated by primary internal fixation, and over half of these were Type III injuries. Their infection rate was low at 4%, and in these patients there was a failure to achieve a stable fixation.

However, none of these series had control groups, and all were retrospective studies;

Chapman and Mahoney (1979) admitted that in 375 open fractures treated conservatively at the same hospital during part of their study period, the infection rate was 2.1%. These infection rates after primary internal fixation are higher than in open fractures treated by conservative methods (Patzakis et al 1974, Gustilo and Anderson 1976).

Although external fixation has been used in the treatment of fractures since the end of the 19th century (Parkhill 1897, Lambotte 1913), it is only during the last two decades that its use has become widespread. These systems of fixation are particularly appropriate in the management of open fractures as they provide stabilisation of bone, while allowing easy access to the wounds.

External fixators have acquired a reputation for causing delayed and non-union (Green 1983). However, in most reports they have been used in severe open fractures which already have a high risk of such problems. The other major problems are pin-track infection or loosening and malunion. Pin-track infection varies in frequency according to definition, but rates of 27% (Court-Brown and Hughes 1982), 30% (Burny 1979) and 42% (Edge and Denham 1981) have been reported for unilateral frames. Similar figures have been seen using double-frame systems (Green and Bergdorff 1980, Edwards 1979). Malunion remains a problem, with up to 55% (Edge

and Denham 1981) and 37.5% (Court-Brown and Hughes 1982) of patients affected. This is mainly due to an unsatisfactory initial reduction, combined with a lack of adjustability of the fixation system.

The dilemma of external fixation is that the degree of rigidity necessary remains undefined. It is known that increasing stability at the fracture retards callus formation, yet even the most rigid external fixators may not stabilise the fracture enough for primary bone union to occur (Green 1983). Although less rigid frames have higher rates of union, infection is more common. Edge and Denham (1981) reported a series of 30 patients with open fractures of the tibia treated by a unilateral frame fixator of their own design : 60% of these patients developed infection at the fracture site. This system is one of the less rigid of those available (Kempson and Campbell 1981). The Vidal-Hoffman apparatus was shown in the same study to be a rigid form of external fixator. Lawyer and Lubbers (1980) used this device to treat 34 patients with open tibial fractures. Their average time to union was longer than that reported by Edge and Denham (1981), but they reported "no major problems" with osteomyelitis.

Perhaps an ideal external fixator would allow rigid stabilisation initially to promote soft tissue healing and possibly to reduce

infection. Moreover, it could be modified in later stages of management to allow some movement at the fracture site in order to stimulate callus formation. De Bastiani et al (1984) have recently described a new fixator which is easily adjustable and which permits rigid immobilisation initially. Once periosteal callus is seen, the fixator can be converted to dynamic fixation by use of its telescopic, sliding bar. This has been called a Dynamic Axial Fixator. Although the initial report is encouraging (De Bastiani et al 1984), the place of this new device in fracture management remains to be clarified.

There is no doubt that stability of the fracture is an important factor in the treatment of established post-traumatic osteitis. Both Burri (1975) and Weber and Cech (1976) stress that the most important factors in the management of infected non-unions are the resection of all necrotic bone and the stabilisation of the non-union by internal or external means. An implant which is still stabilising the fracture should be left in situ (Burri 1975). When infection occurs in open fractures treated by primary internal fixation, it is nearly always possible to eradicate the osteitis and achieve bone union if the principles laid down by Burri (1975) and Weber and Cech (1976) are adopted (Rittmann et al 1979).

The hypothesis that stability will affect the outcome favourably in post-traumatic osteitis has been studied experimentally by Rittmann and Perren (1974). They examined the effect of infection on bone healing by performing tibial osteotomies in sheep and fixing them with plates of differing rigidity, giving three degrees of stability. Staphylococci in high concentrations ($3.1 - 12.9 \times 10^{10}$) were injected into the osteotomy one week after surgery. The inoculation was repeated until infection ensued. Despite the small number of animals (only 19 sheep in total) they were able to conclude that rigid fixation of the osteotomy offered favourable conditions for bone healing. It was therefore advantageous to leave a stabilising implant in place when infection was present. They felt that the advantage of the stabilising effect of implants outweighed the possible disadvantages of a foreign body effect. Although this study supports the concept that stability is of value in treating established osteitis, it throws no light on the question of whether stabilising a contaminated open fracture will prevent the development of infection.

There has been only one experimental study which tests the hypothesis that rigid fixation of a contaminated open fracture reduces its susceptibility to infection. Friedrich and Klaue

(1977) performed stable (plate) and unstable (intramedullary rod) fixation of fractures of rabbit tibiae. These tibiae were then inoculated with a standard strain of Staphylococcus aureus in a concentration of 10^5 organisms. They stated that abscesses, sinuses and sequestra developed in 45% of animals with unstable fixation of fractures; in contrast, osteitis evident clinically did not occur after rigid fixation ($p<0.001$).

Although the work of Friedrich and Klaue (1977) initially appears convincing, they admitted that even with stable fixation using plates there was radiological and histological evidence of infection. This statement was not discussed any further and they did not state how many animals displayed such evidence of infection. However, if stable fixation is to prevent infection there should be no evidence of osteitis by <u>any</u> criteria. It is thus difficult to accept their conclusion that stable fixation reduces the susceptibility of the fracture to infection when histological and radiological signs of infection were seen in animals with plate fixation.

It is clear that despite the confident claims by some surgeons (Muller et al 1979, Chapman 1982, Allgower and Border 1983), there is as yet no convincing evidence to support the hypothesis that stable fixation of a contaminated

open fracture reduces its susceptibility to infection.

Effect of Antibiotics

The value of antibiotics in the treatment of open fractures still remains in doubt. While some workers advocate the routine use of antibiotics (Patzakis et al 1974, Gustilo and Anderson 1976), others argue that they are unecessary (Muller et al 1979, Rittmann et al 1979).

Before discussing the available evidence, the dynamics of wound infection and how antibiotics might alter this process will be outlined. It is clear that most open fractures will be significantly contaminated with bacteria by the time the patient reaches hospital. Gustilo and Anderson (1976) reported that 70% of initial wound cultures in such patients were positive, and Patzakis and Ivler (1977) found that 62.1% of open fractures had positive wound cultures before treatment.

The size of bacterial inoculum is of importance in the development of wound infection. Using quantitative microbiological assessments of traumatic wounds of the hand and forearm, Cooney et al (1982) found that sepsis was unlikely to occur with less than 10^5 organisms per gram of tissue. All but two of their eighteen patients

with more than 10^5 organisms developed clinical wound infection. The smaller study of Marshall et al (1976) confirms that 10^5 organisms per gram of tissue is the critical size of the inoculum.

The other important feature in the development of wound infection is the time interval between injury and surgical treatment, for there is a time-dependant relationship with bacterial proliferation. Robson et al (1973) found that the mean time from injury to surgical treatment in wounds with less than 10^2 organisms per gram of tissue was 2.2 hours; in those wounds containing 10^2 -10^5 organisms per gram the mean time from injury to surgery was 3.0 hours, and in those wounds with more than 10^5 organisms the mean delay was 5.17 hours. The work of Edlich et al (1969) also suggests this time-dependant relationship of bacterial proliferation; this time factor is important because once the colony count is greater than 10^5 organisms per gram of tissue, wound infection is likely.

Another important factor is that there is a "decisive period" in the first four hours after inoculation, during which the eventual outcome is determined. Miles, Miles and Burke (1957) found that, using skin wounds in guinea pigs, there was a finite time after bacterial inoculation during which the final extent of the infection was determined. If host defences were inhibited by

local ischaemia or by generalised dehydration shock, they noted that the size and extent of the local infection was increased. This "enhancement" was maximal when the enhancing agent was used in the first two hours after inoculation, and had effectively disappeared by four to five hours after inoculation. They also demonstrated that when antibiotics were used to suppress infection, the maximal effect was observed when the antibiotic was given before bacterial contamination. However, the antibiotic effect became progressively less, so that if the first dose was delayed until four hours after inoculation, no beneficial effect was seen.

This work was confirmed by Burke (1961) who also inoculated skin wounds in guinea pigs with Staphylococcus aureus. The initial dose of antibiotic was given at differing times, from one hour before to six hours after inoculation. Burke confirmed that there is a short period when infection can be suppressed by the appropriate use of antibiotics. Antibiotics have the maximum effect if given before bacteria gain access to the tissues; this effect became progressively less as the initial dose was delayed. If the initial dose was not given until three or more hours after inoculation, no effect was seen.

There is little comparable data for bone infection, although Bowers et al (1973) described

an experimental model of osteitis in the intact femur of dogs. After preparing a cortical bone window, Staphylococci were injected into the marrow cavity and the cortex replaced. They demonstrated that when cephaloridine was given pre-operatively the wounds became sterile and infection did not develop. If the antibiotic was given six hours or more after inoculation, infection occurred uniformly. However, no attempt was made to study the effect of antibiotics given in the first few hours after inoculation - a situation of considerable clinical relevance. There is no experimental study which evaluates the effect of antibiotics in preventing infection in a fracture contaminated with bacteria, and then relating this to the timing of the initial dose of antibiotic.

The evidence available from experimental studies on skin wounds (Miles, Miles and Burke 1957, Burke 1961) suggests that if antibiotics are to have any effect on a contaminated wound, they must be given as soon as possible after inoculation. Ideally, the antibiotic should be present in the tissues before contamination - a situation denied to the orthopaedic surgeon treating an open fracture.

The data from clinical studies does not completely resolve the question of efficacy. In their series, Rittmann et al (1979) used

penicillin/streptomycin therapy initially, but found it to have no effect on the development of osteitis and discontinued its use. The AO group feel that antibiotic treatment in open fractures has no value and that the disadvantages outweigh the possible advantages (Muller et al 1979).

Gustilo and Anderson (1976) advocate the routine use of intravenous antibiotics in high dosages. Although their overall infection rate was low, there was no control group which did not receive antibiotics. Hence it is difficult to be certain that antibiotic therapy contributed to the clinical result. Patzakis et al (1974) did include a control group which received no antibiotic. They found an infection rate of 13.9% in the control group, 9.7% in the group receiving penicillin and streptomycin, and 2.3% in those patients who received cephalothin. The difference between the control and the penicillin and streptomycin groups was not significant statistically; but there was a significant difference between the control and the cephalothin treated groups. In contrast, Bergmann (1982) in a smaller study, was unable to demonstrate that antibiotics had any significant effect on the infection rate after open fracture.

AIMS OF STUDY

This experimental study had the following objectives:

1. To develop an animal model of a contaminated open fracture, in which osteomyelitis can be induced reliably and reproducibly.

2. To use this model to assess the effect of stability on the development of osteomyelitis. This involved stabilising the fracture with a relatively rigid fixation system (Dynamic Compression Plate) or with unstable fixation (loose intramedullary rod). Infection was diagnosed by clinical, radiological, microbiological and histological methods.

3. To use this model to assess the effect of antibiotic therapy in reducing infection rates, with particular reference to the timing of initial antibiotic therapy in the first four hours after inoculation. Only one fixation system was used in this phase of the study - using the model with the highest osteomyelitis rates in the preceeding experiments without antibiotics.

Selection of an experimental model

The choice of a suitable animal model for this study was dictated by two main considerations:

1) The need to produce a standard fracture

and stabilise it with the two fixation systems (compression plate or intramedullary rod).

2) The ability to induce osteomyelitis reliably in the animal selected.

Various experimental models have been previously described for the study of fracture healing. Large animals have the advantage that the operative techniques are easier. In contrast, the cost of small animals is much less.

The typical features of primary bone healing have been reported in dogs (Schenk and Willenegger 1967, Olerud and Danckwardt-Lilliestrom 1968, Schenk 1978), sheep (Rittmann and Perren 1974) and rabbits (Rahn et al 1971, Ashurst et al 1982). In all these studies, fracture healing occurred by Haversian re-modelling as osteons crossed the fracture line. This healing required stable internal fixation with contact between the bone ends. Ashurst et al (1982) also described the pattern of fracture healing in rabbits with unstable fixation of experimental tibial fractures: when flexible plastic plates were used, fracture union was associated with external callus formation.

The pattern of healing in experimental fractures stabilised with an intramedullary rod has been studied in both rats (Kernek and Perry 1981, Molster et al 1982) and in rabbits

(Ellsasser et al 1975, Wang et al 1981, Dekel et al 1981). All these workers confirmed that the method was technically easy to perform and that fracture healing occurred by external callus formation.

It is evident that it is possible to create standard fractures of the rabbit tibia. These fractures can be stabilised with either a compression plate or an intramedullary rod; fractures heal by primary bone union with the former, while in the latter healing is by external callus formation. The use of the rabbit as an experimental animal has a further advantage in that the structure of cortical bone is very similar to that in humans (see Figures 1.2 and 1.3). In contrast, there is a virtual lack of Haversian systems in small rodents (Ashurst et al 1982).

Inducing osteomyelitis reliably in an experimental animal is difficult. Scheman et al (1943) described a model of osteomyelitis in rabbits. An inoculum of Staphylococcus aureus together with a sclerosing agent (sodium morrhuate) was injected into the tibial metaphysis; they reported that characteristic radiological and clinical signs of osteomyelitis developed. These signs were not seen when either Staphylococci or sodium morrhuate was injected alone.

FIGURE 1.2 : Rabbit cortical bone. The Haversian systems are clearly seen, H and E. Magnification x 200.

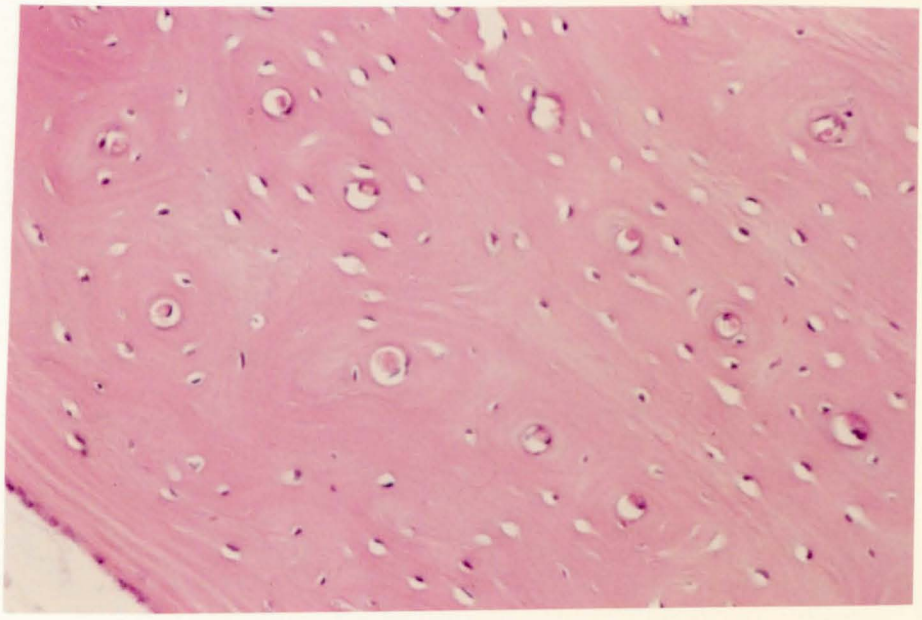
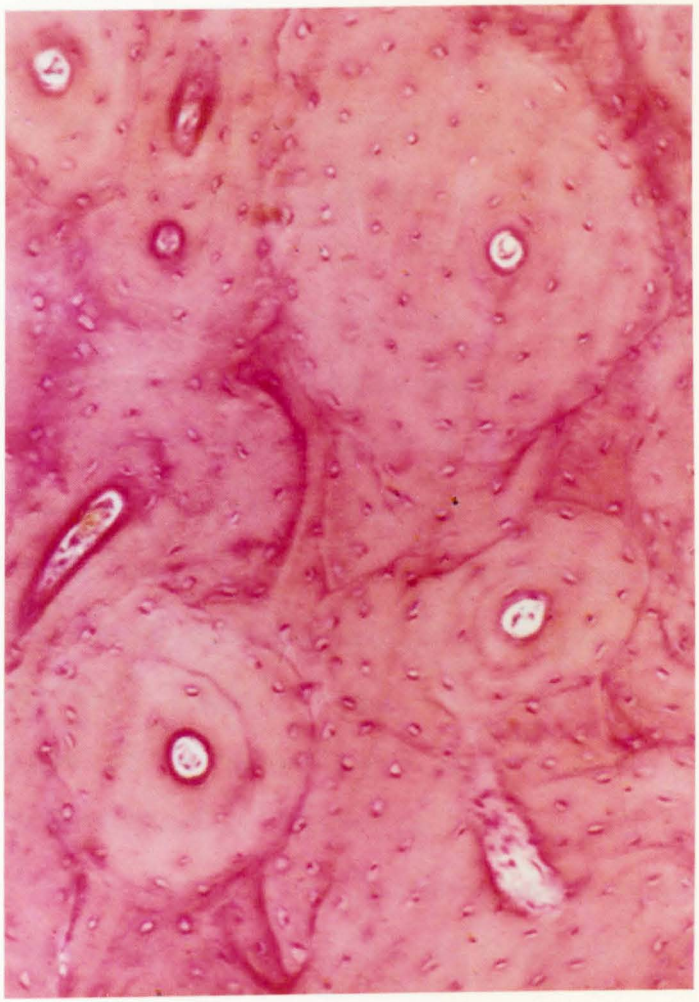


FIGURE 1.3 : Human cortical bone. H and E. Magnification x 200. (Reproduced, with permission, Craigmyle 1975).



This experimental model of Scheman et al has been refined by Norden (1970), Crane et al (1977) and Mayberry-Carson et al (1984). Using a standard inoculum of $3x10^6$ Staphylococci together with 0.1ml 5% sodium morrhuate, these workers reported that osteomyelitis can be reliably induced in 60% to 90% of animals. Crane et al (1977) described in detail the correlation between the histological and radiological signs of infection. Moreover, they confirmed that the radiological signs of osteomyelitis in the rabbit are similar to those seen in man.

Rissing et al (1985) have demonstrated that this model can also be used in rats. They were able to induce osteomyelitis in 51% of animals when Norden's technique was repeated exactly, and this was increased to 81% when a drill was used to breach the tibial cortex. The increased infection rate may be due to additional trauma to the bone, caused by the high speed drill.

This animal model has been further modified by Dekel and Francis (1981). The basic technique was unchanged, but Staphylococci of a different phage type and a different sclerosing agent were injected. Using an inoculum of 10^7 Staphylococci together with 0.4ml 3% sodium tetradecylsulphate, osteomyelitis was found in over 60% of their animals. This suggests that neither the sclerosing agent nor the phage type of

Staphylococcus aureus is specific for osteomyelitis in rabbits.

In all these studies, a sclerosing agent was used to produce vascular thrombosis. In an attempt to reproduce a situation of septic micro-embolisation, analagous to that proposed in man, Deysine et al (1976) described a technique of injecting Staphylococci into the nutrient artery. A mixture of 0.1ml barium sulphate with an inoculum of 10^6 Staphylococci was injected into the nutrient artery of the canine tibia. This produced obliteration of small arterioles with infected micro-emboli. Although many dogs died in the acute phase of osteomyelitis, some developed the typical appearance of chronic osteomyelitis (Deysine et al 1983).

Both the studies using a sclerosing agent and those using injection of septic micro-emboli were essentially experimental models of acute haematogenous osteomyelitis. There have been few attempts to develop a suitable animal model of post-traumatic osteomyelitis after a fracture.

Andriole et al (1973) have described a technique of producing closed, midshaft fractures of the rabbit tibia. A hole was then drilled in the upper tibia, through which an inoculum of 10^8 Staphylococci was injected. The fracture was then stabilised with an intramedullary rod, introduced through the drill hole. They reported that 88% of

animals developed radiological and microbiological evidence of chronic osteomyelitis. No signs of osteomyelitis were observed when 10^8 Staphylococci were injected into the medullary cavity without an accompanying intramedullary rod.

Although Andriole et al (1973) produced osteitis reliably, without a high mortality rate, this is not a true experimental model of post-traumatic osteomyelitis; there was no wound over the fracture and Staphylococci were not introduced directly into the fracture site. The experimental model of Friedrich and Klaue (1977) approximates more closely to an open fracture. In their study, rabbit tibiae were fractured and stabilised with either a compression plate or an intramedullary rod. Staphylococci, in an inoculum of only 10^5 organisms, were then introduced into the fracture site and the wound closed. They observed osteomyelitis in these animals, but were vague on the precise rate of infection. Their work, as suggested before, raises many questions.

An experimental model of a contaminated open fracture has also been developed in guinea pigs by Passl et al (1984). The femur was fractured under direct vision and stabilised with an intramedullary rod; the fracture site was inoculated with 10^4 Staphylococci and the wound closed. Although only five animals out of seven in this group survived, all showed signs of

chronic post-traumatic osteomyelitis.

Rittmann and Perren (1974) induced post-traumatic osteomyelitis in sheep, after stabilisation of a tibial osteotomy with a compression plate. They injected Staphylococci directly into the fracture site. Infection was seen in all their animals, but this was very artificial because large numbers of bacteria ($>10^{10}$) and repeated inoculations were used.

Based on the available data it was decided to use a fracture of the rabbit tibia as the experimental model for my study.

PLAN OF THESIS

My work will be considered in two phases: firstly, the effect of stability and secondly, the effect of antibiotics in preventing infection of experimental open fractures. Aspects of the methods which are common to all the experiments will be described first.

The first phase of this study was to test the hypothesis that stable fixation of a contaminated fracture reduces its susceptibility to infection. Three preliminary studies were performed before the main experiment. Firstly, the pattern of fracture healing was determined with both stable (compression plate) and unstable (intramedullary rod) fixation, each without bacterial inoculation. Secondly, a mechanical study was performed on cadaveric rabbit tibiae to establish the difference in stability between plate and rod fixation. Thirdly, a model of post-traumatic osteomyelitis was developed which was used in the definitive study.

In the second phase of the work, the fixation system with the highest rate of osteitis was used to examine the effect of antibiotics. Prior to this, the pharmacokinetics of cephradine in rabbits were investigated to confirm an appropriate dosage schedule. The effect of

antibiotics was examined by giving one dose only of cephradine, at varying intervals in relation to inoculation of the fracture site with bacteria.

The implications of this work for clinical practice will be discussed and the possibilities for further research using these techniques will be reviewed.

CHAPTER 2

GENERAL METHODS

2.1 Experimental animals

There were 148 male New Zealand white rabbits used in this experiment. All weighed over 3.5kg, the average weight being 3.92kg. The animals were kept in individual cages and fed on a commercial rabbit diet (SG1), with unlimited fresh water.

2.2 Operating environment.

All operations were performed in a conventional operating theatre. Incoming air was filtered primarily by Super V Filters and secondarily by Autoroll Standard Filter Medium; these have an efficiency of 95% against BS2831 test dust No2. There were 18-20 air changes per hour.

The same theatre team (surgeon, assistant and anaesthetic technician) took part in all operations. A standard anaesthetic technique was used, with a gas induction by mask using oxygen, nitrous oxide and Halothane. A flow rate of one litre/minute was maintained for oxygen and nitrous oxide, whilst the Halothane was gradually increased to a maintenance level of 2.5%-3%. The anaesthetic was terminated at the end of the procedure by withdrawing Halothane and nitrous oxide, while maintaining the rabbit on oxygen via

the mask until consciousness was regained. All the animals were given one intramuscular dose of 0.25ml buprenorphine (0.3mg/ml) as post-operative analgesia.

2.3 Bacteria

The organism used for the majority of the study was a Staphylococcus aureus, of a phage type 29, isolated from a patient with chronic osteomyelitis (this organism is hereafter referred to as COM 1). During the pilot study of the osteitis model, three other strains of Staphylococcus aureus were tested and further details are given later.

The COM 1 organism has been investigated in the Department of Biochemistry, University of Nottingham. It is characterised by an increased ability to survive in anaerobic conditions, when compared with other isolates of Staphylococcus aureus (Coleman et al 1983).

The organisms were stored on nutrient agar slopes (Oxoid) at a temperature of 4°C. The inoculum for experimental work was prepared by sub-culturing in 10mls nutrient broth (Oxoid) overnight at 37°C. It was then diluted in sterile buffered phosphate saline to give the required final inoculum (in 0.5mls saline). After each inoculum was prepared in this way, surface viable counts were performed on CLED agar plates to

determine the number of colony forming organisms (CFU's) per millilitre.

2.4 Sacrifice

All animals were sacrificed at 12 weeks after operation with an intravenous injection of 2.5ml Euthatal (sodium pentobarbitone 200mg/ml). The skin of the operated leg was then shaved and cleaned with Hibisol (2.5% chlorhexidine and 70% isopropyl alcohol). The wound was re-opened using aseptic technique and the tibia freed from its soft tissue attachments and removed. A swab from around the implant was taken for microbiological assessment. The distal one centimetre of the tibia was removed with a saw to allow access to the intramedullary rod. The tibia was then placed in 10% formol calcium (see Chapter 2.5. 4).

2.5 Assessment

The same methods of assessment were used for all animals in all phases of the study. There were four methods of assessment:

2.5.1) Clinical assessment:

The animals were inspected for general signs of infection (malaise and weight loss) at weekly intervals after surgery. The wound was inspected at 48 hour intervals for the first week, and weekly thereafter. The presence of any erythema, bruising or swelling was recorded and

any wound discharge was assessed by culture.

2.5.2) Radiological assessment:

Radiographs were obtained immediately after operation and at two-weekly intervals thereafter until the animal was sacrificed. The rabbits were anaesthetised for subsequent films with an intramuscular injection of 0.75ml Hypnorm (0.315mg fentanyl and 10mg fluanisone per ml). The anaesthetised animal was laid prone on the cassette with both legs fully extended and in the same rotational alignment. A postero-anterior film was taken with the x-ray beam centred on the base of the tail (technical data: single phase, four pulse generator; 80mA, 64kv, 0.15 sec at a focal distance of 100cms).

A lateral radiograph was also obtained after sacrifice and removal of implants and soft tissue, before de-calcification. Eight explanted tibiae were placed on each cassette in the lateral position (technical data: three phase, six pulse generator; 40mA, 54kv, 0.13 sec at a focal distance of 100cms). All radiographs were obtained using CEA RPL film, mounted in a Kodak X-omatic Fine cassette.

The radiographs were assessed by two observers - one clinician (the candidate) and one independent radiologist. The radiologist had no knowledge of the details of the study so that his assessment was therefore "blind". The radiographs

were assessed using seven criteria. These are based on the radiological descriptions of osteomyelitis in rabbits (Crane et al 1977) and sheep (Rittmann and Perren 1974); these are the same features as seen in human osteomyelitis. A reproduction of the assessment sheet is shown in Figure 2.1 and each finding was defined as follows:

1) Fracture line : this was either present (+) or absent (-). The point in time at which the distinct line disappeared was recorded.

2) Callus : this was graded on a four point ordinal scale from absent (-) to extensive (+++). This description referred to new bone formation at the fracture site.

3) Periosteal reaction : this was graded on a four point ordinal scale from absent (-) to extensive (+++). This reaction was defined as diffuse new bone spreading sub-periosteally along the diaphysis. It was graded according to the distance the reaction spread along the diaphysis.

4) Osteolysis : this was defined as resorption at the fracture site and/or either lytic areas appearing around the implants or elsewhere in the bone. It was recorded as being present (+) or absent (-).

5) Sclerosis : this was defined as increasing density of new bone, without increasing extent. It was recorded as being present (+) or absent

RABBIT TIBIA - RADIOLOGICAL ASSESSMENTS

RABBIT No: .....

DATE OF OPERATION: .....

The criteria are assessed as either present (+) or absent (-) except 'callus' and 'periosteal reaction' which are assessed on a four-point score: '-' indicates the absence of a finding, up to '+++' for extensive findings.

DATE OF X-RAY	Fracture line	Callus	Periosteal reaction	Osteolysis	Sclerosis	Involucrum	Soft tissue swelling
Initial post-op							

Date of sacrifice: .....

Final radiological pattern of union

Primary intention
Secondary intention
Non-union

Infected/not infected

FIGURE 2.1 : The radiological assessment form

(-).

6) Involucrum : the presence (+) or absence (-) of an involucrum was recorded.

7) Soft tissue swelling : this was measured at the level of the tibio-fibular synostosis. The baseline width of the leg was either the smaller of the measurements on the first and last radiographs of the fractured limb or the width of the normal leg. An increase of width of 3mm on the operated side was regarded as significant. This was recorded as either present (+) or absent (-).

Based on the overall series each observer made two further comments on each animal:

A) Radiological pattern of union. "Healing by primary intention" was defined as union with no instability callus and only minimal fixation callus, no rounded edges at the fracture site or fracture gaps. "Healing by secondary intention" was defined as union by external callus of uneven structure, with widening of the fracture gap. "Non-union" was defined as no sign of bony union between the fragments.

B) Whether there was any radiological sign of osteomyelitis.

2.5.3) Microbiological assessment:

Any wound discharge was sent for

bacteriological assessment. After sacrifice of each animal the tibia was explanted under strict aseptic conditions. A culture swab (cotton wool - Exogen) was taken from around the implant for culture. All swabs were immediately placed in transport medium (Amies) and taken to the laboratory. Swabs were inoculated onto blood agar (Oxoid) and incubated overnight at 37°C. Any isolates were identified and if confirmed as Staphylococcus aureus, were sent for bacteriophage typing by the Public Health Laboratory Services.

2.5.4) Histological assessment:

After explantation the tibia, including implants, was fixed by placing it in 10% formol calcium for two weeks. After removal of implants a lateral radiograph was obtained, as previously described. Three blocks were cut from the tibia using a hacksaw (see Figure 2.2 - Blocks A,B,C). The proximal edge of each block was marked with a one millimetre saw cut to maintain orientation.

The three blocks were de-calcified using EDTA for a minimum of eight weeks, with five changes of EDTA during that time. The first batch of eight tibiae was assessed by slab radiographs at two-weekly intervals to check that the de-calcification was complete. These showed that this was complete by eight weeks and further specimens were not radiographed.

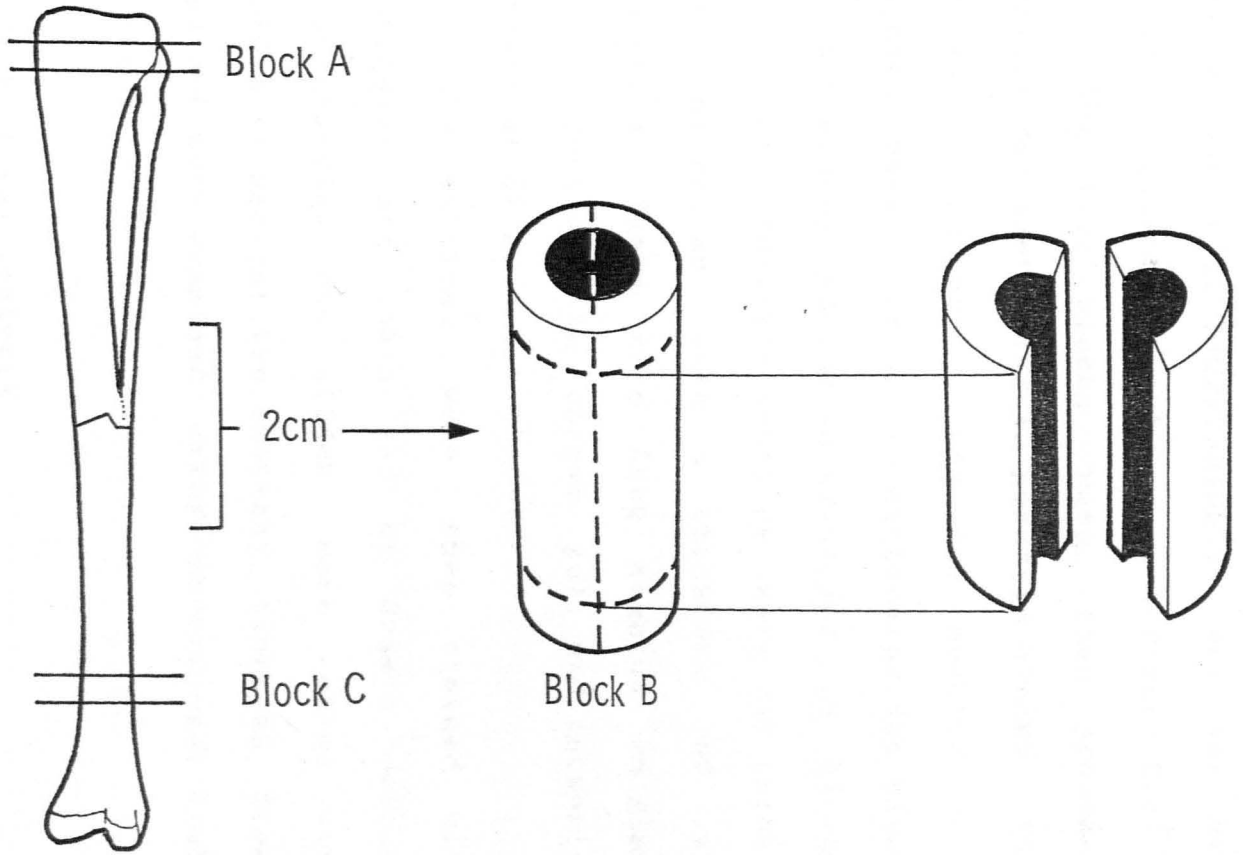


FIGURE 2.2 : Three blocks were cut from each tibia as shown. Block B was further divided to cut transverse sections from each end and a longitudinal section across the fracture site.

After de-calcification, block "B" was further divided to allow transverse sections to be cut from the proximal and distal ends, while the remainder was split longitudinally and one half used for longitudinal sections (see Figure 2.2).

The five blocks were then processed overnight for sectioning in paraffin blocks. They were embedded in paraffin wax and mounted on a hardboard base. Prior to sectioning the blocks were softened by soaking in glycerine in alcohol for one hour. Multiple sections were cut using a rotary microtome with a thickness of six micrometres. These were then mounted on glass slides, treated with chrome gel, and incubated overnight at 37°C.

The sections were then stained with heamatoxylin and eosin and by Gram's method. During staining the slides were coated with celloidin to prevent the sections floating free. The slides were examined using conventional light microscopy.

2.6 Statistical analysis

With the small number of animals in each group, statistical analysis was performed using Fisher's exact probability test (Armitage 1971). Where appropriate groups could be amalgamated, to form groups of sufficient numbers, the Chi-square test was used.

CHAPTER 3

FRACTURE HEALING AFTER FIXATION WITH COMPRESSION PLATES AND INTRAMEDULLARY RODS

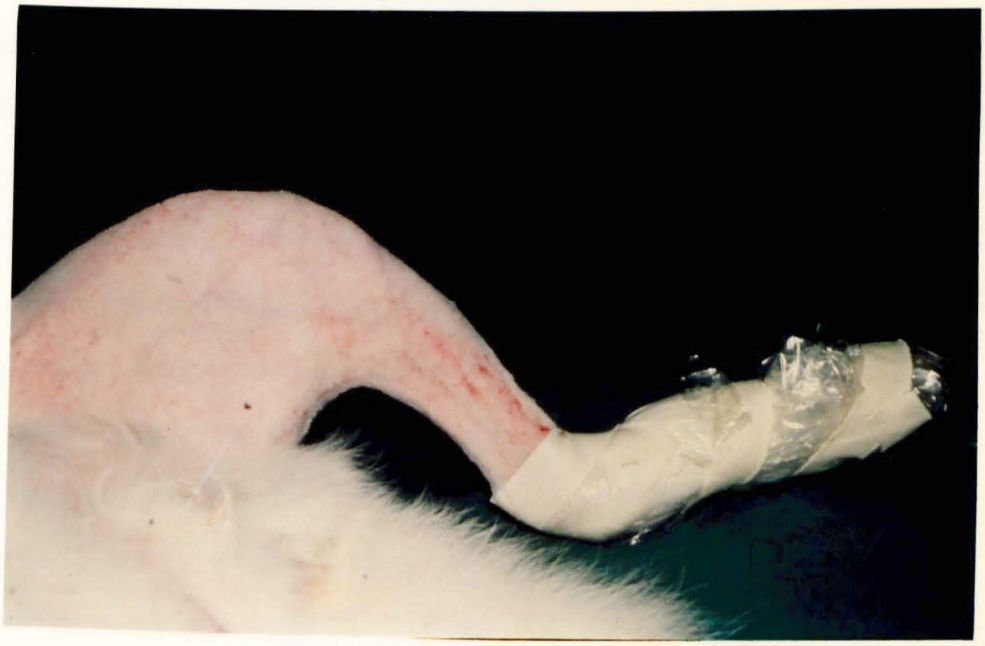
3.1 Methods :

The fractures were stabilised with either a compression plate (stable fixation) or an intramedullary rod (unstable fixation). The first part of the operation was the same with both methods of fixation. The rabbits were anaesthetised, using the technique described in Chapter 2.2, and the left hind-leg shaved from ankle to groin. The paw was wrapped in a cellophane bag, sealed to the skin with elastoplast (see Figure 3.1); this prevented blood from contaminating the fur, thus minimising local irritation. The animal was then placed prone on the operating table.

The skin was cleaned with Hibisol (2.5% chlorhexidine and 70% isopropyl alcohol) and the leg draped with sterile sheets. The operations were performed under sterile conditions. The medial surface of the tibia was exposed through a long antero-medial incision. The deep fascia was divided from its anterior attachment to the tibia, taking care to avoid stripping the periosteum.

If the animal was in the "stable" group, the fracture was fixed with a six-hole 2.7mm AO dynamic compression plate (DCP) of a thickness of 2.0mm (Straumann UK Ltd: Catalogue No 244.06). The design of the screw holes causes traction on

FIGURE 3.1 : The left hind-leg shaved and wrapped. The animal lies prone on the operating table with the medial surface facing the surgeon.



the plate, and thereby axial compression on the bone, when the screws are tightened (Perren et al 1969). This principle is illustrated in Figures 3.2 and 3.3. A special drill guide (see Figure 3.4) was used to standardise the position of the screw holes. The gap of 12.7mm between the central screw holes ensures that these screws will lie eccentrically within the central holes of the dynamic compression plate. As they are tightened, axial compression across the fracture site results, as shown in Figure 3.3. The plates were made of stainless steel (type AISI 316L), conforming to BS3531 : Part 1.

The drill guide was placed in position on the medial surface of the tibia and a 2.0mm bit used to drill the holes. The distal hole was drilled first and a 2.0mm rod inserted through the guide into the hole, maintaining the position of the drill guide. The proximal hole was drilled next and a second rod inserted (see Figure 3.5). The remaining holes were then drilled. The drill bit was cooled continuously with saline. A thread was then tapped in each drill hole with a 2.7mm AO hand tap.

The dynamic compression plate was then bent to conform to the contour of the bone in the chosen position. The plate was pre-bent according to the technique of the AO group (Muller et al 1979). The periosteum was incised transversely,

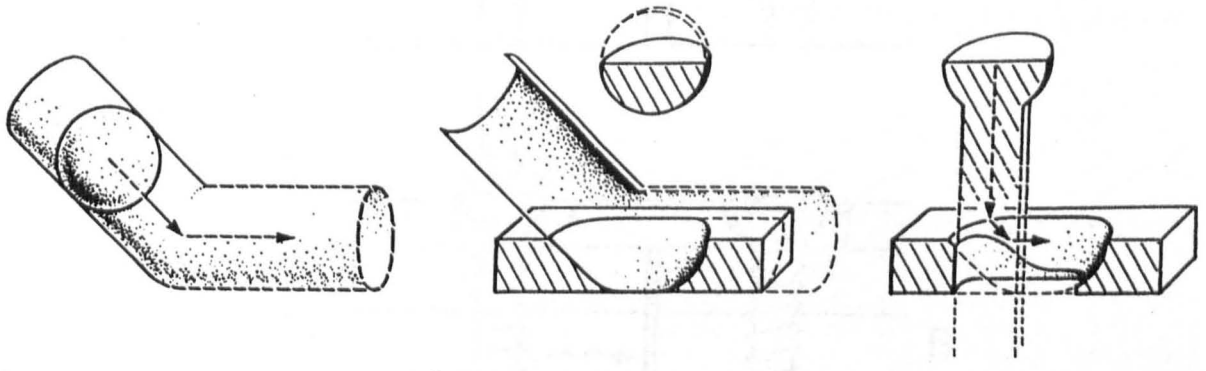


FIGURE 3.2 : The screw hole of the dynamic compression plate (DCP): the geometry of the screw hole causes axial compression in the bone when the spherical screw head glides down the sloped plane. (a) "Ball-glide-principle": a ball is guided in a sloped cylinder (load track) and horizontal cylinder (glide plane). (b) The screw hole is spherically formed. The screw hole is composed of two cylindrical segments. (c) Correlation between screw and screw hole in the longitudinal section. (Reproduced, with permission, from Rittmann and Perren 1974).

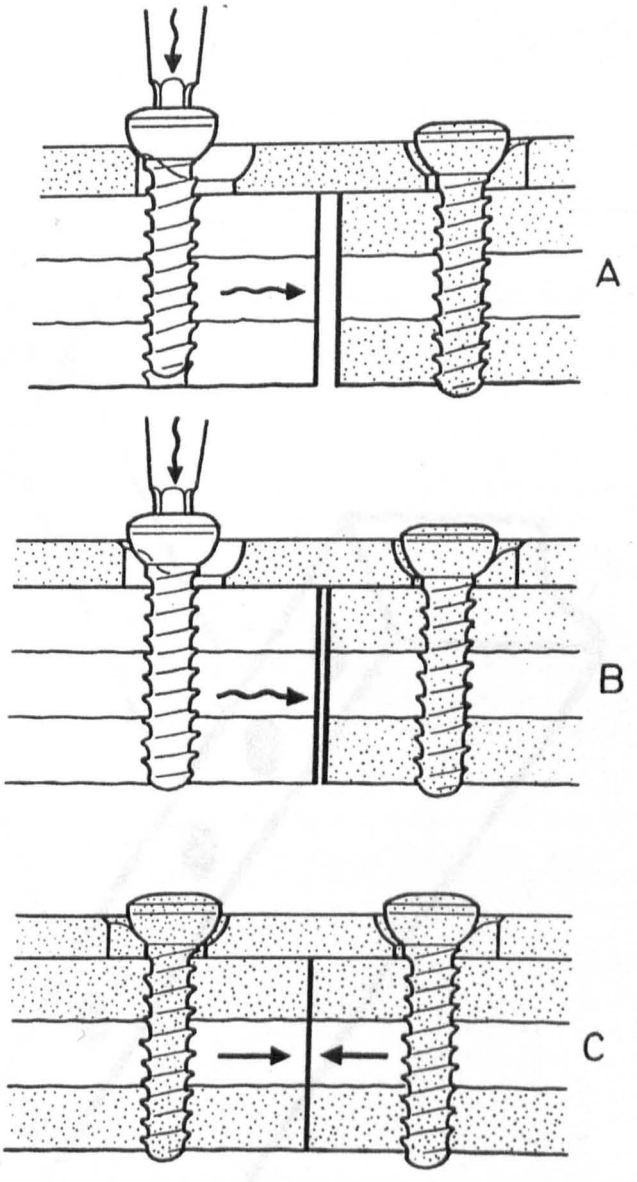


FIGURE 3.3 : Axial compression on bone by the use of a dynamic compression plate. The sloped plane in the holes makes it possible to exert axial compression on the osteotomy. With the first screw the implant is fixed to one main fragment. Driving the second screw home (A to C) along the sloped plane of the screw hole moves the second main fragment (⇝) towards the osteotomy. After complete adaption, an axial force (→) causes traction on the plate and compression on the osteotomy. (Reproduced, with permission, from Rittman and Perren 1974).

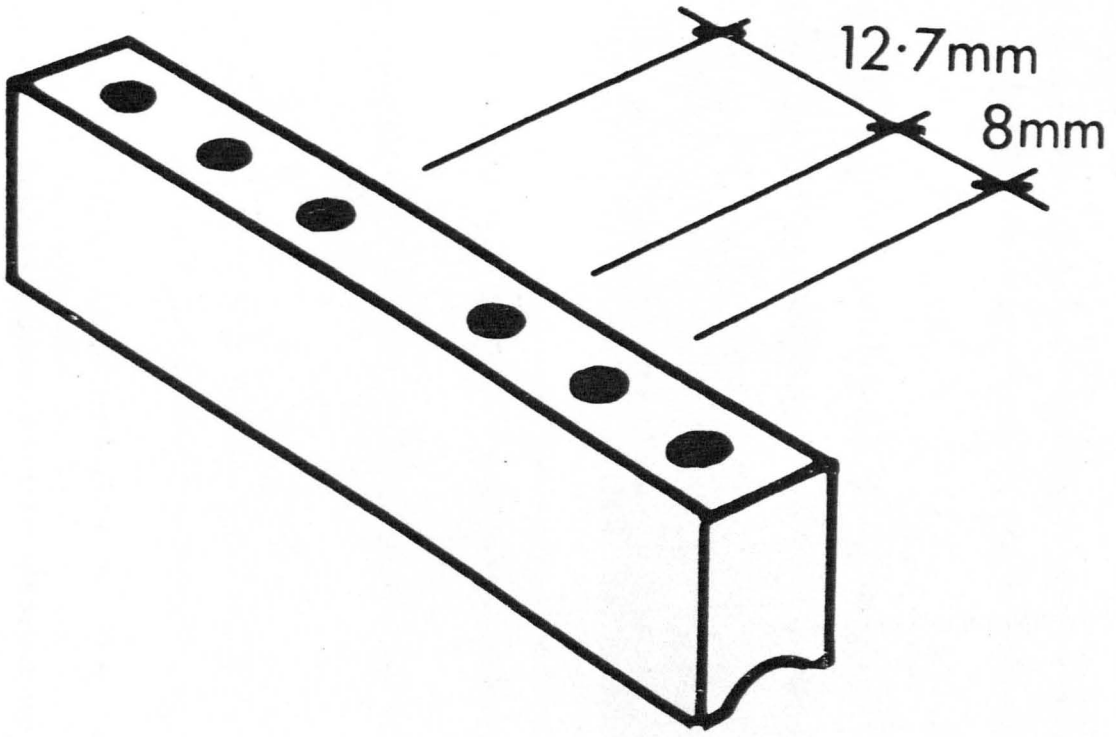
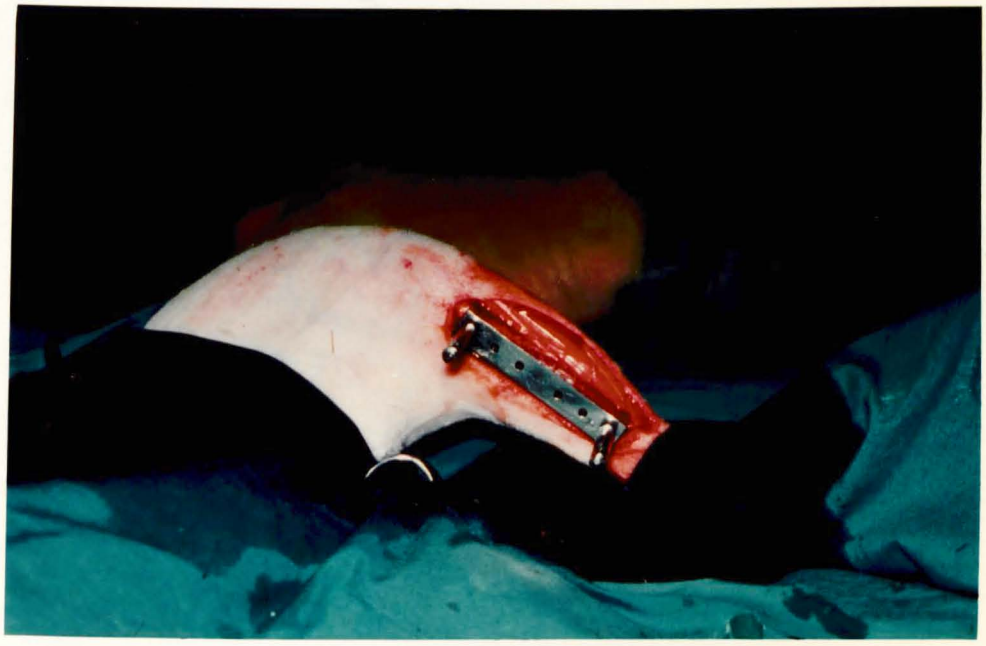


FIGURE 3.4 : Diagram of the drill guide. (Reproduced, with permission, from Ashurst et al 1982).

FIGURE 3.5 : The drill guide held in position by the two rods, after drilling the proximal and distal screw holes.

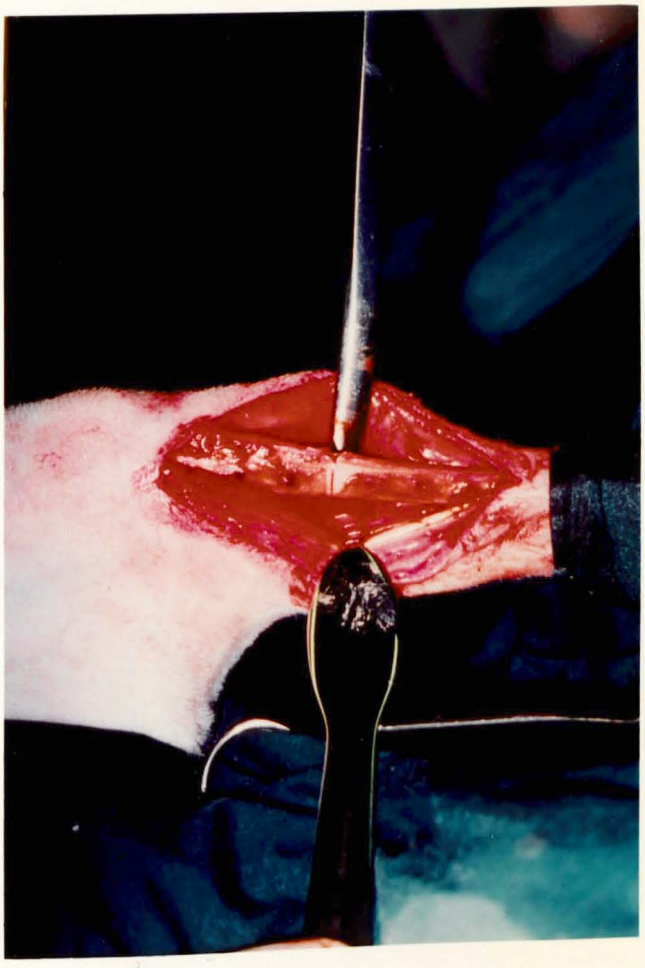


in the centre of the gap between the central screw holes. A small transverse cut was made just through the medial cortex of the tibia at this point, using an oscillating saw with a blade 100 micrometres thick (see Figure 3.6). The saw blade was cooled continuously with saline.

The bone was then fractured using the C-clamp and the fracture device illustrated in Figures 3.7 and 3.8. This exerts pressure at three points on the bone : at each end of the fracture device on the medial surface (via the two rods) and immediately opposite the saw cut on the lateral border (via the C-clamp). As the clamp is tightened, the pressure increases until the bone fractures (see Figure 3.9).

The fracture was held in position and the 2.7mm dynamic compression plate applied, carefully avoiding any soft tissue damage laterally or posteriorly. The plate was fixed to the bone using AO 2.7mm cortical screws of a length of 12mm. Prior to finally tightening the screws, a 23G needle was introduced through the skin and its tip only laid in the gap created by the saw cut (see Figure 3.10). As the screws are tightened the fracture closes with the axial compression generated by the DCP effect (Perren et al 1969). The needle tip lay in the gap of the saw cut and did <u>not</u> prevent an anatomical reduction of the fracture. This needle was the route used to

FIGURE 3.6 : The medial surface of the tibia after drilling all six holes. The transverse saw cut is midway between the central drill holes, near the tip of the upper retractor.



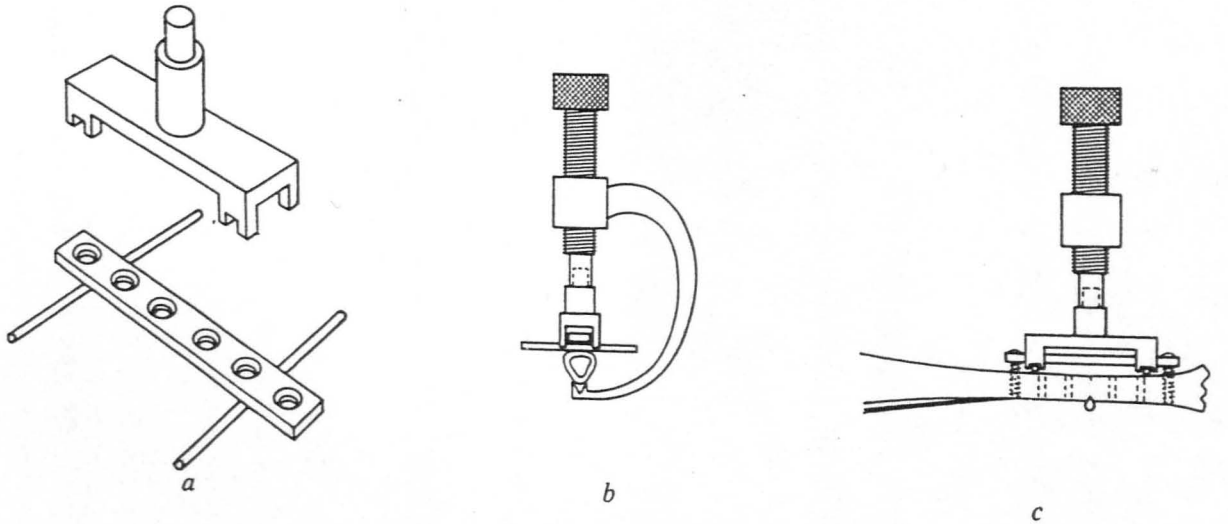


FIGURE 3.7 : The fracture device and C-clamp. (a) The fracture device and position of the rods in relation to the plate. (b) Transverse view of the device, rods and C-clamp on the bone. (c) Lateral view of the position of the instruments on the bone before fracture. (Reproduced, with permission, from Ashurst et al 1982).

FIGURE 3.8 : The fracture device, rods and C-clamp in position.

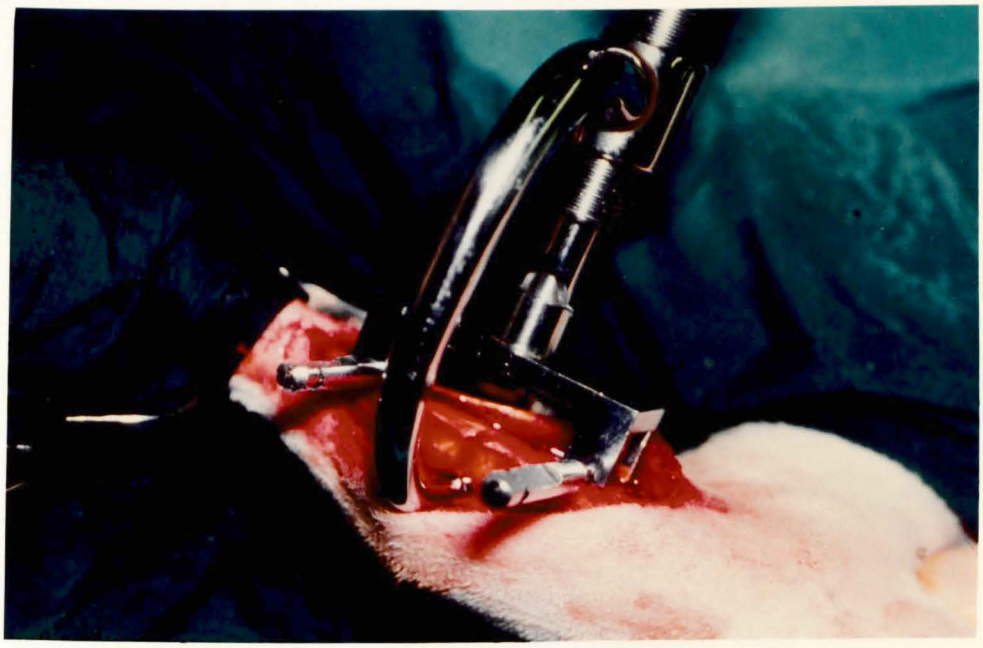


FIGURE 3.9 : The reproducible midshaft fracture of the tibia.

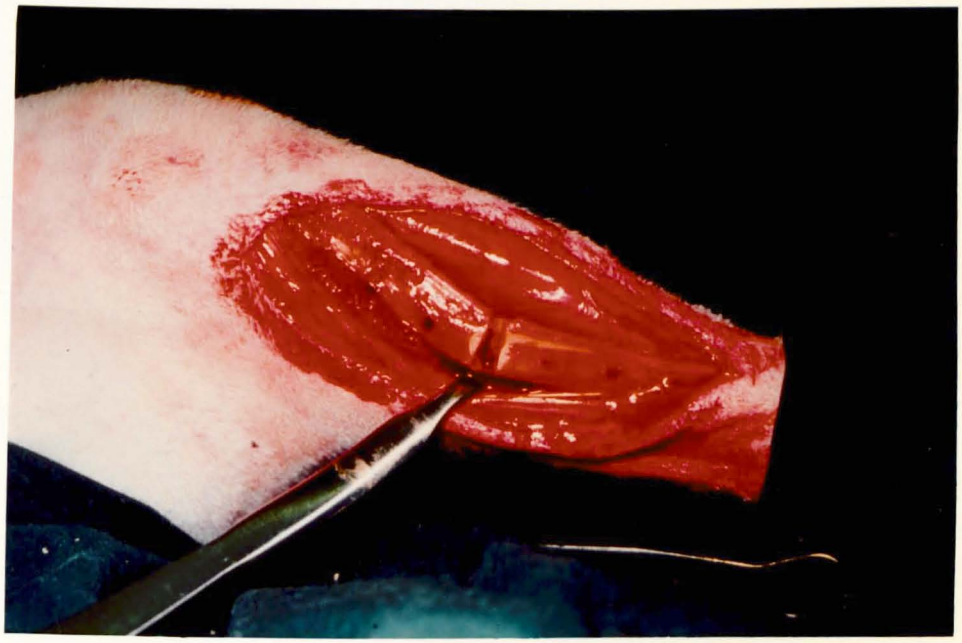
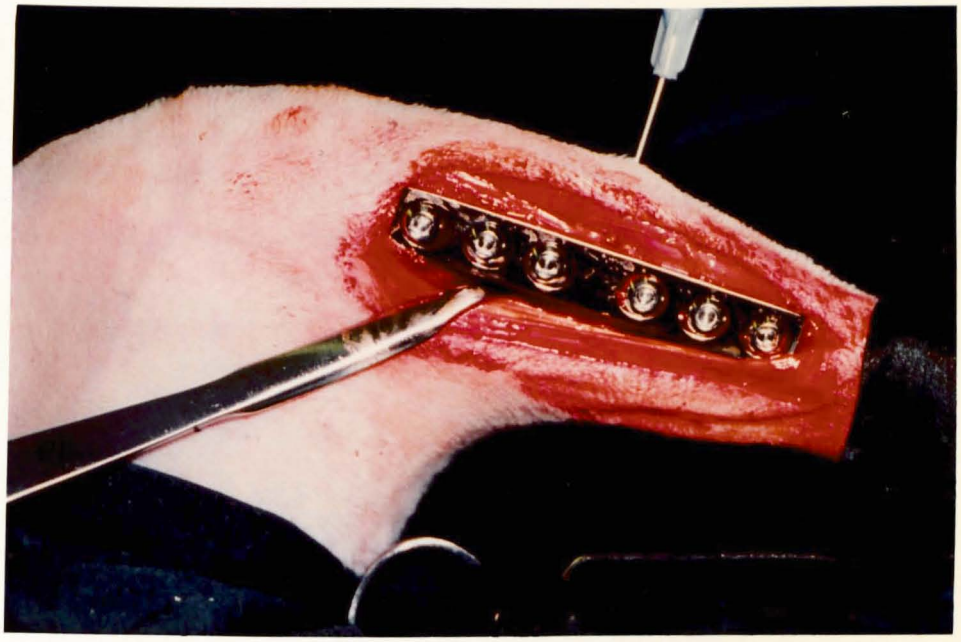


FIGURE 3.10 : The fracture reduced and held with the dynamic compression plate. The needle tip lies in the saw cut beneath the plate.



inoculate the fracture site with bacteria in later phases of the study.

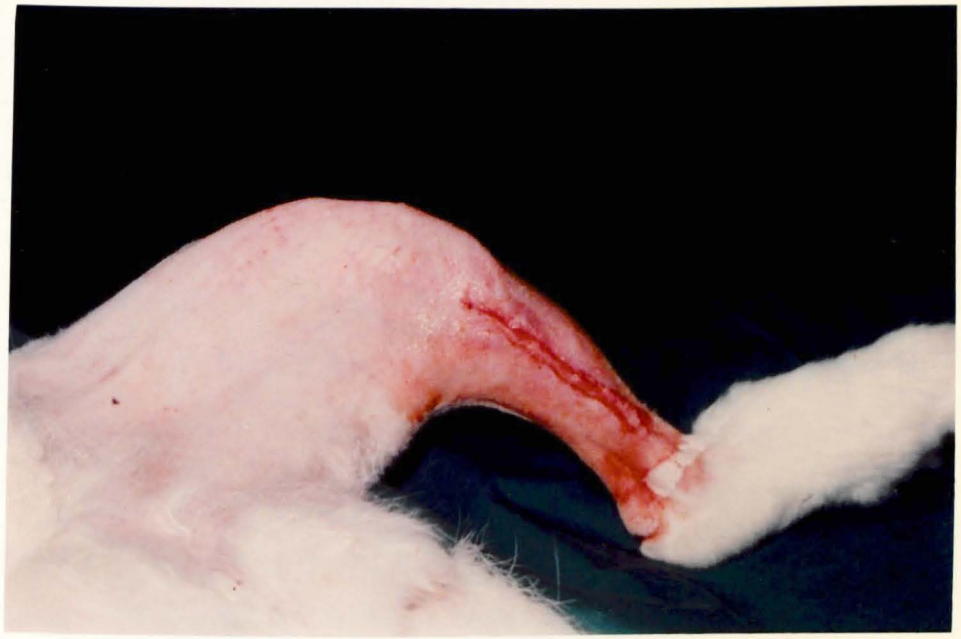
The superficial fascia was closed over the plate with interrupted sutures of 3/0 Dexon (Davis and Geck Ltd). The skin was sutured with a 4/0 Dexon subcuticular suture, taking care to bury the knots under the skin. The wound was then cleaned of any blood and protected with Nobecutane spray only (see Figure 3.11).

If the animal was in the "unstable" group, the fracture was fixed with a 3.0mm diameter intramedullary rod (Straumann GB Ltd : Catalogue No 292.30). These were also made of stainless steel type AISI 316L and were cut down to a length of 100mm prior to autoclaving. This size of rod fitted loosely into the intramedullary canal of the tibia in male rabbits, over 3.5kg in weight, and was long enough to reach the distal metaphysis (Ashurst 1984).

After exposing the medial surface of the tibia, as described previously, the periosteum was incised transversely at the mid-shaft. A transverse cut was made just through the medial cortex of the tibia using an oscillating saw with a blade 100 micrometres thick. The saw blade was cooled with saline.

A longitudinal skin incision was then made over the patellar tendon and the tendon split longitudinally in the line of its fibres. The

FIGURE 3.11 : The final appearance of the wound after closure.



100x3mm rod was mounted in a hand introducer and introduced into the medullary canal though the tibial tuberosity. The site of entry was outside the synovial membrane of the knee joint (see Figure 3.12). Care was taken to introduce the rod in the line of the tibial shaft to avoid perforation of the metaphyseal cortex. The rod was introduced into the medullary canal for a distance of three to four centimetres, so that the tip remained proximal to the site of fracture.

The C-clamp and fracture device were then placed in position, as described above, and the clamp tightened until the bone fractured (see Figure 3.13). The rod was then advanced across the fracture site into the distal shaft. A small punch was used to tap the rod home, flush with the proximal surface of the tibial tuberosity. A 23G needle was introduced through the skin and its tip placed against the rod in the fracture gap.

The patellar tendon was repaired with interrupted 3/0 Dexon sutures and the superficial fascia closed over the fracture with 3/0 Dexon. Both wounds were closed with a subcuticular suture of 4/0 Dexon, care being taken to bury all knots beneath the skin. The leg was cleaned to remove any blood stains and the wounds protected with Nobecutane spray.

In both groups, after skin closure, 0.6ml of saline was injected through the 23G needle into

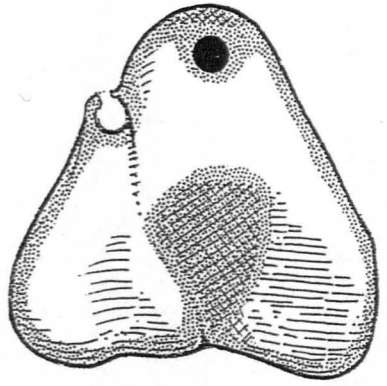
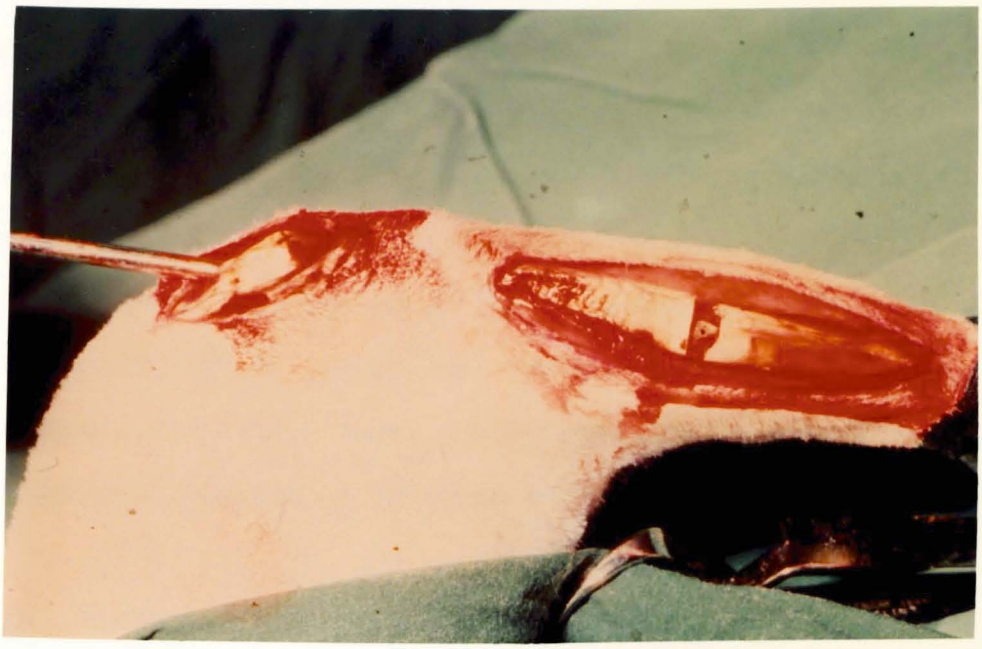


FIGURE 3.12 : Diagram of tibial plateau to show site of insertion of rod. The hatching marks the line of attachment of the synovium. The rod is inserted anteriorly into the tibial tuberosity, outside the joint cavity.

FIGURE 3.13 : The intramedullary rod is introduced into the proximal tibia through the tibial tuberosity. The tip of the rod can be seen at the fracture site.



the fracture site. The needle was then withdrawn. A radiograph was then obtained, as described in Chapter 2.5.2, while the animal was still anaesthetised. The rabbits were then returned to their cages for post-operative recovery. No cast immobilisation was used and unrestricted weight bearing was allowed.

The rabbits were all assessed using the protocols laid down in Chapter 2.5 and were sacrificed twelve weeks after operation, using the technique described in Chapter 2.4.

3.2 Results :

There were 23 rabbits in the experiment. The fracture was fixed with a dynamic compression plate in 11 animals ("stable" group); in one rabbit the tibia fractured extensively during plating and this animal was immediately sacrificed and excluded from the study group. A loose-fitting intramedullary rod was used to stabilise the fracture in 12 rabbits ("unstable" group); in two of these the distal tibia shattered on insertion of the rod and these animals were immediately sacrificed and excluded from the study group. These technical failures in the rod-fixed group were both in rabbits weighing just over 3.5kg. In subsequent experiments, care was taken to ensure that all animals were well over 3.5kg in

weight.

There were therefore ten animals in each group who completed the experimental protocol.

Clinical assessment :

In both groups there was evidence of slight swelling and bruising of the operated leg which persisted for 48-72 hours before resolving. In the "unstable" group callus was palpable around the fracture site for four to six weeks.

Rabbits in the plate-fixed group were invariably taking weight on the operated leg by the end of the first post-operative week. However, in the rod-fixed group, weight -bearing on the operated leg was not seen until about two weeks after surgery.

There were only two minor complications. In the first animal operated on, interrupted skin sutures were used. This rabbit nibbled at the wound, which required re-suturing. Thereafter, a sub-cuticular Dexon was used to close all skin wounds. Marked rotational instability of the distal tibia was observed in one rabbit in the rod-fixed group. The limb was therefore immobilised in an above knee plaster-of-Paris cast for the first two weeks after operation. On removal of the cast, recovery was uneventful.

No other complications were seen. In particular, no clinical evidence of wound infection was seen in any of the animals.

Microbiological assessment :

No organisms were isolated from any of the culture swabs taken from around the implants.

Radiological assessment :

The assessment of the radiographs by the candidate and the independent radiologist showed good conformity. There was agreement on the pattern of fracture healing in every animal. There were only minor time differences in regard to the appearance or disappearance of a particular radiographic sign.

The fracture line was invisible on the immediate post-operation radiograph of all animals in the plate-fixed group; the cut made in the medial cortex with the oscillating saw was usually visible (see Figure 3.14).

In one rabbit in the "stable" group, loosening of the distal screws was observed on the radiograph obtained after two weeks. However, the alignment and position of the fracture remained satisfactory and the animal was not in any distress. This fracture healed with radiological features of external callus formation.

The remaining nine rabbits, treated with a dynamic compression plate, showed a characteristic pattern of bone healing on the serial radiographs. There was minimal periosteal reaction on the cortex opposite the plate between two and six weeks (see Figure 3.15). This is due to the

FIGURE 3.14 : A radiograph of a fracture fixed with a dynamic compression plate, obtained immediately after operation. The fracture line cannot be seen, although the saw cut is just visible (opposite the arrow).

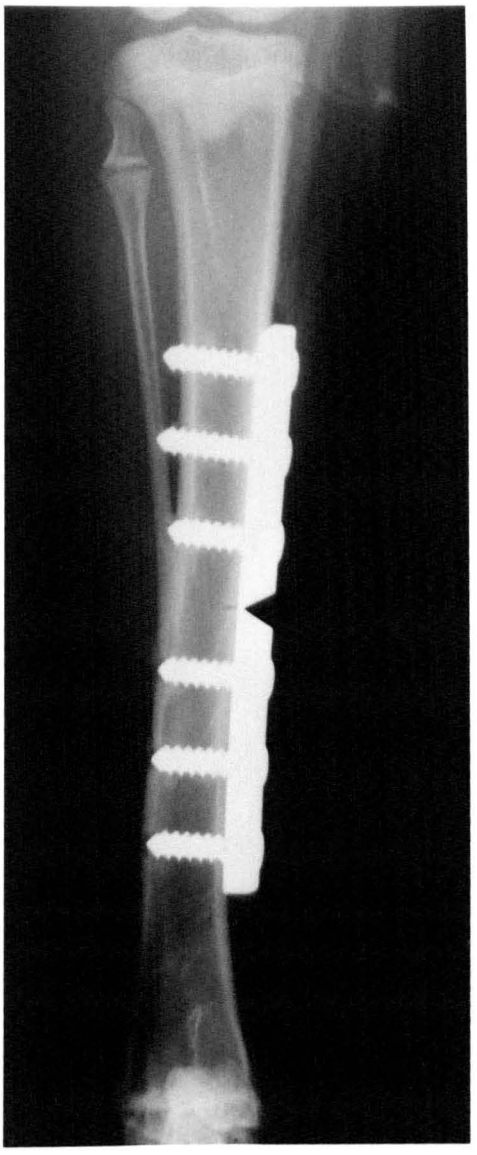
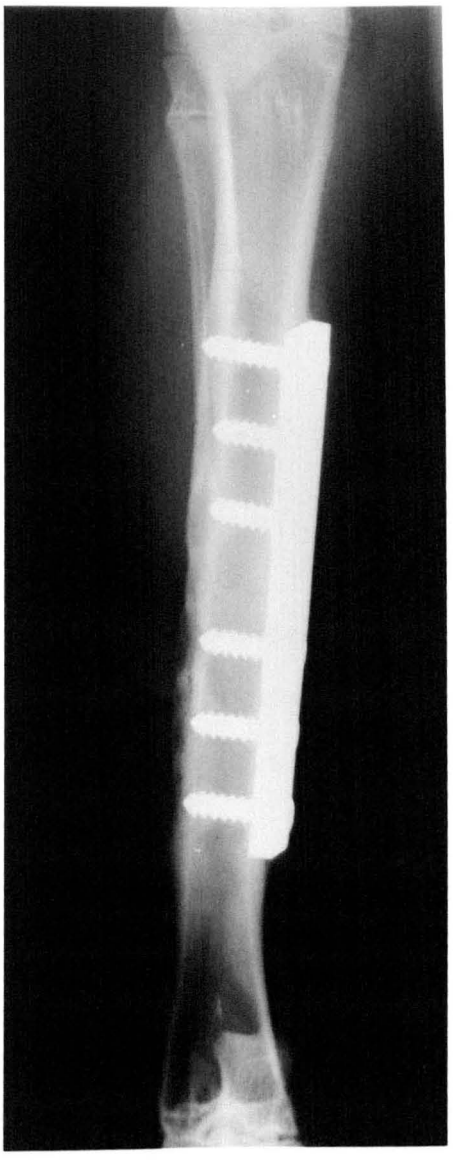


FIGURE 3.15 : A radiograph obtained four weeks after fixation with a dynamic compression plate. There is periosteal reaction where the screws have pierced the lateral cortex.



raising of the periosteum by the screws as they penetrate the far cortex (Ashurst, 1984). In one rabbit this minimal periosteal reaction persisted for ten weeks, but in all the others it had resolved by six weeks. No external callus formation was seen at the fracture site in any animal.

On the radiographs obtained at four weeks, there was a zone of intra-medullary sclerosis in the region of the fracture site. This resolved quickly and the normal tubular appearance of the diaphysis was restored by six weeks. These characteristic changes are clearly seen in the serial radiographs shown in Figure 3.16.

Minimal soft tissue swelling was observed radiographically, in four animals only, at two weeks, but not thereafter. The radiological pattern of bone healing seen in these nine animals is similar to that reported by Rahn et al (1971) and Ashurst et al (1982) in their descriptions of primary bone union.

A fracture stabilised with an intra-medullary rod is shown in Figure 3.17. This appearance was typical of the immediate post-operative radiograph of the ten animals in this "unstable" group; the loose fit of the rod within the medullary canal is clearly demonstrated.

A characteristic radiological pattern of

FIGURE 3.16 : Serial radiographs after fixation with a dynamic compression plate, obtained at two-weekly intervals. There is healing of the fracture without external callus formation.

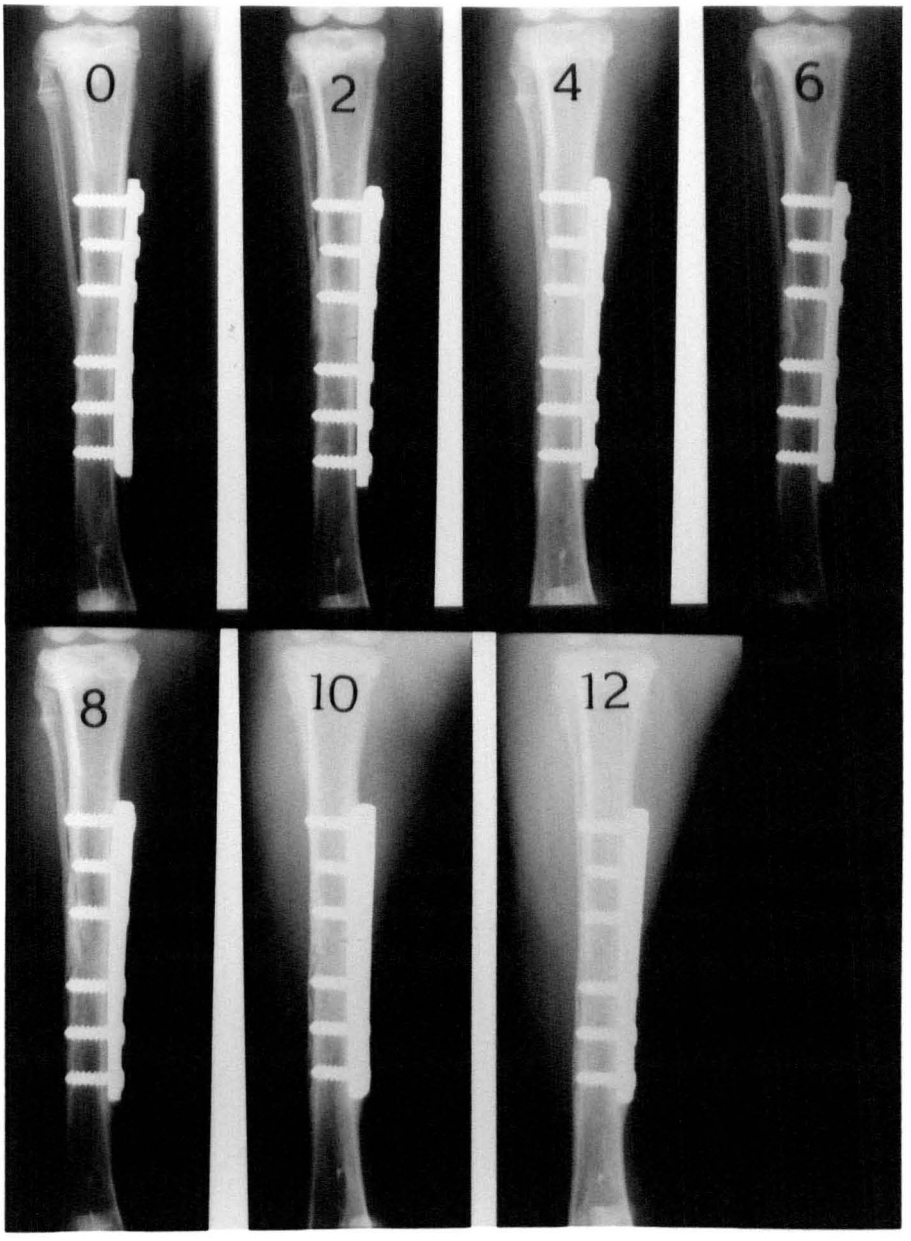
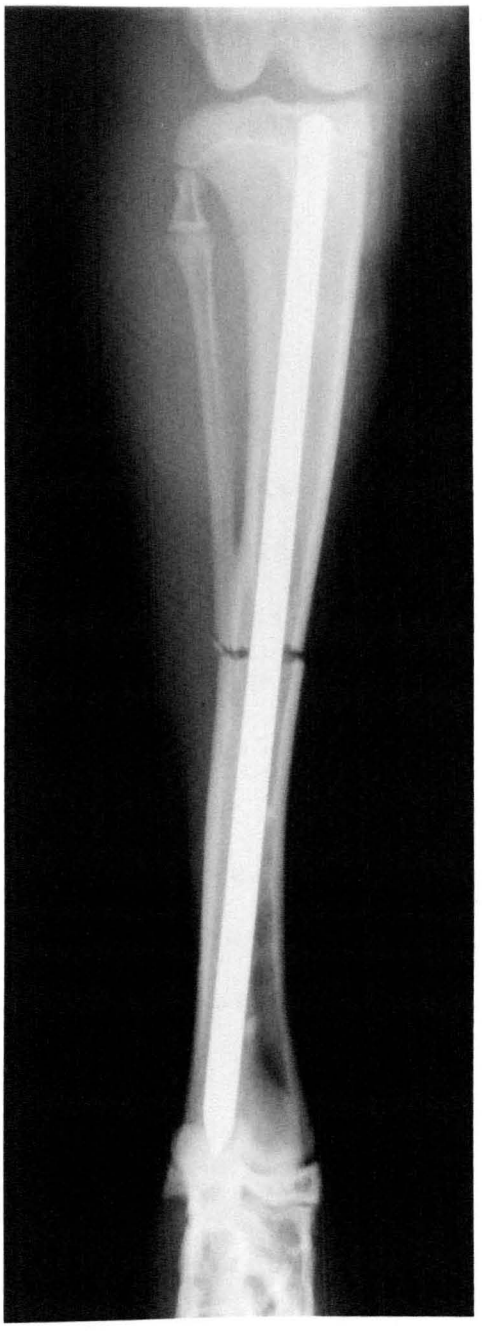

0
2
4
6
8
10
12

FIGURE 3.17 : Fracture stabilised with an intramedullary rod. The rod fits loosely into the medullary canal, with minimal contact on the endosteal surface.



healing was seen in all the ten rabbits. The fracture line persisted as a distinct line until four weeks in all animals; in four rabbits the fracture line was still visible at six weeks. External callus formation at the fracture site was first seen at two weeks, reaching its maximal extent by four weeks. The callus persisted until ten to twelve weeks, by which time re-modelling had occurred. This characteristic pattern of bone healing by external callus formation is shown on the serial radiographs in Figure 3.18.

In all ten animals in the "unstable" group the fracture healed by external callus formation; in the nine animals in the "stable" group in whom the fixation was satisfactory, healing was by primary bone union. This is clearly seen in Figure 3.19, which shows lateral radiographs, obtained after sacrifice and implant removal, of both a rod-fixed fracture and a plate-fixed fracture. The rod-fixed fracture had healed with external callus; but the plate-fixed fracture has healed with the typical radiological appearance of primary bone union.

Histological assesment :

Similar histological features were seen in all nine rabbits in the "stable" group, whose fractures had healed with radiological features of primary bone union. All showed a relative

FIGURE 3.18 : Serial radiographs taken after fixation with an intramedullary rod, obtained at two-weekly intervals. The fracture has healed by external callus formation.

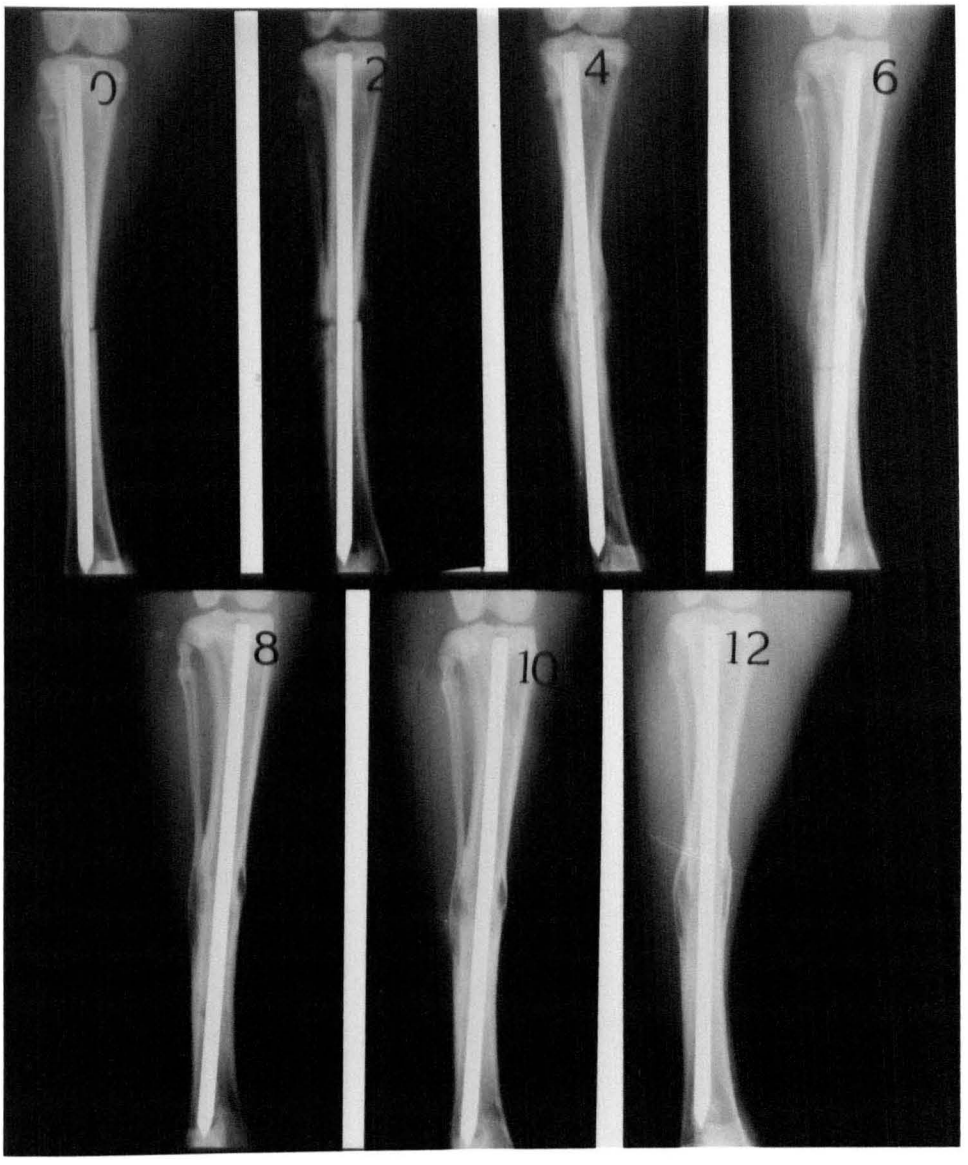
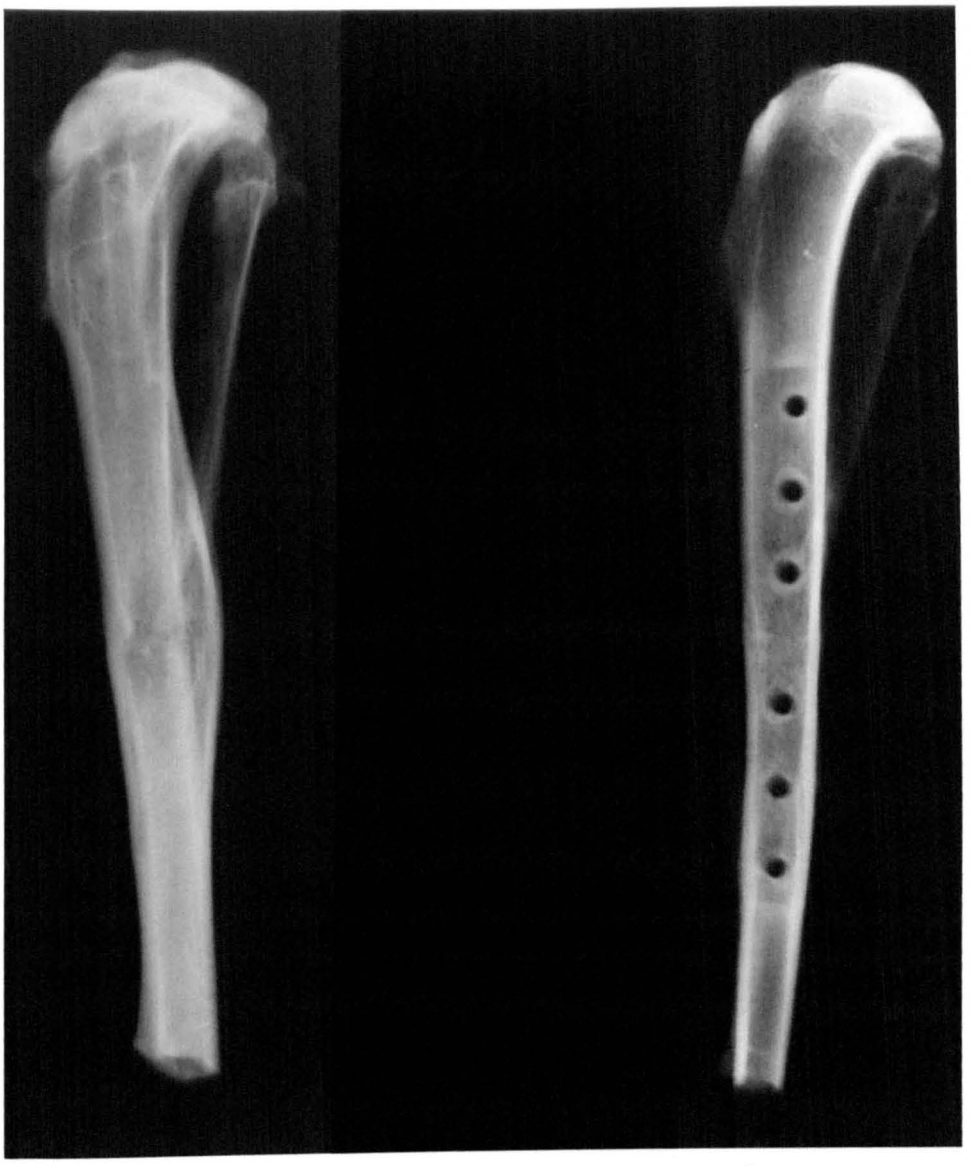

0
2
4
6
8
10
12

FIGURE 3.19 : Lateral radiographs obtained after sacrifice and removal of implants. (a) The fracture fixed with an intramedullary rod has healed by external callus. (b) The fracture fixed with a dynamic compression plate has healed by primary bone union.



a b

osteopenia of the cortical bone immediately under the plate, when compared with normal cortex. This is illustrated in Figure 3.20, which may be compared to normal rabbit bone seen in Figure 1.3.

Periosteal reaction and new bone formation was limited to around the plate and through the screw holes. In four rabbits, areas of cortical necrosis were identified by the typical appearance of lacunae empty of osteocytes. These areas of bone necrosis were limited to the cortex, deep to the periosteal new bone at the edge of the plate (see Figure 3.21).

The fracture site was difficult to identify because of the lack of external callus. However it was often possible to identify the saw cut made in the medial cortex (beneath the plate). The typical features of gap healing were readily identifiable (see Figure 3.22).

In two rabbits it was possible to identify the site of the fracture in the cortex opposite the saw cut. Minimal periosteal new bone is seen and there is direct bridging of cortical bone by osteoid remodelling (see Figure 3.23).

This histological appearance of bone union without periosteal callus and with gap healing in places is in accord with the previous descriptions of primary bone union in the rabbit (Rahn et al 1971, Ashurst et al 1982).

In contrast, the fractures in the "unstable"

FIGURE 3.20 : Longitudinal section of the cortex immediately beneath the plate. There is osteopenia and the appearance is similar to cancellous bone (p - position of plate). H and E. Magnification x 40.

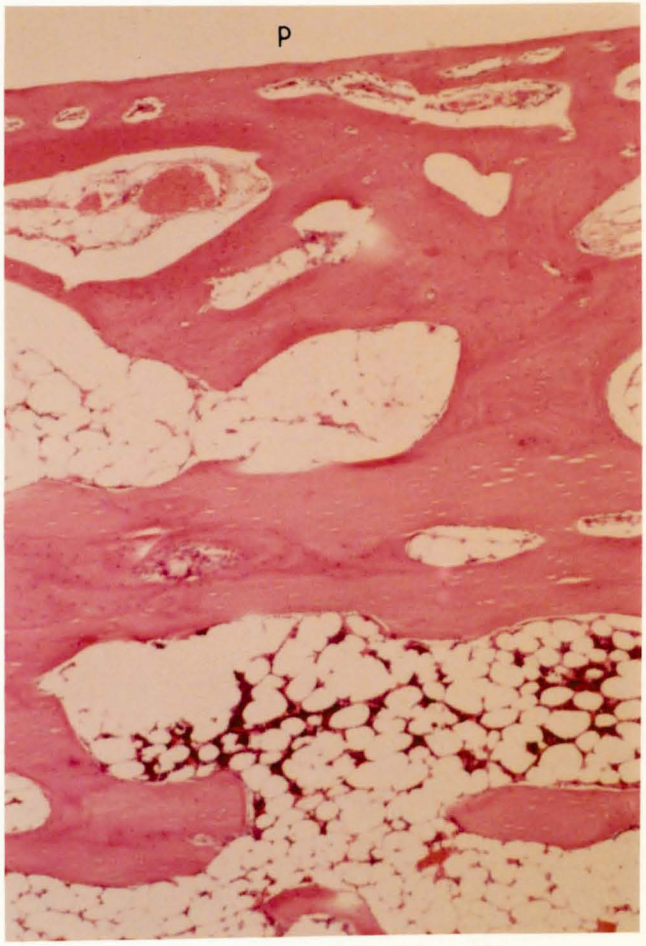

P

FIGURE 3.21 : Transverse section of the cortex at the edge of the plate. There is a thin layer of periosteal new bone (nb) beneath the plate (p). Deep to this, the bone is avascular for some distance. H and E. Magnification x 100.

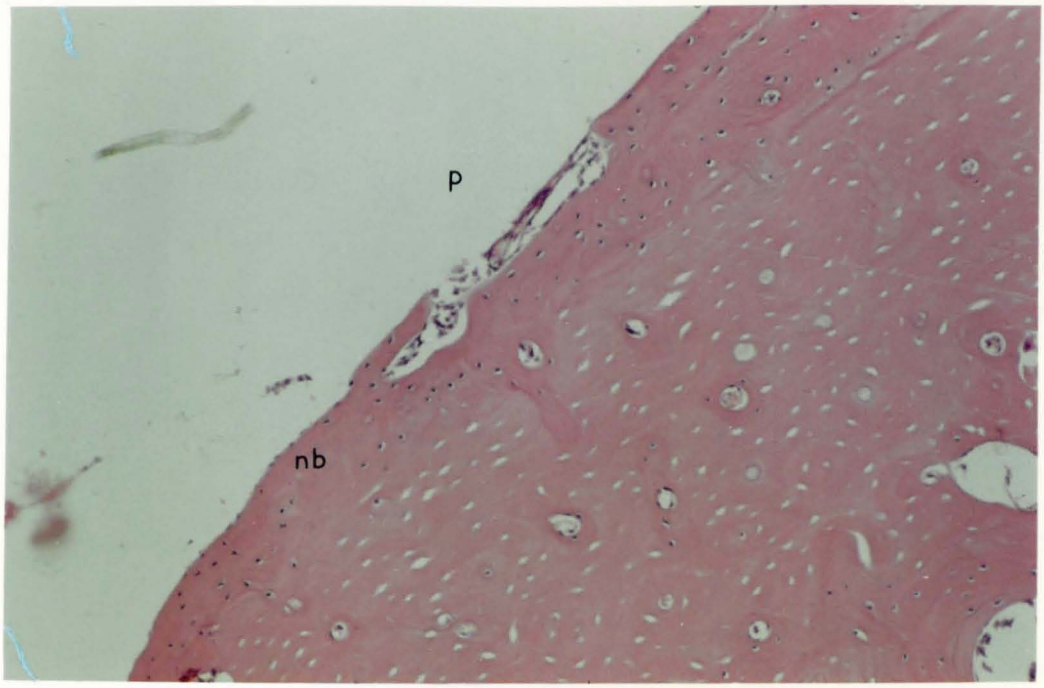
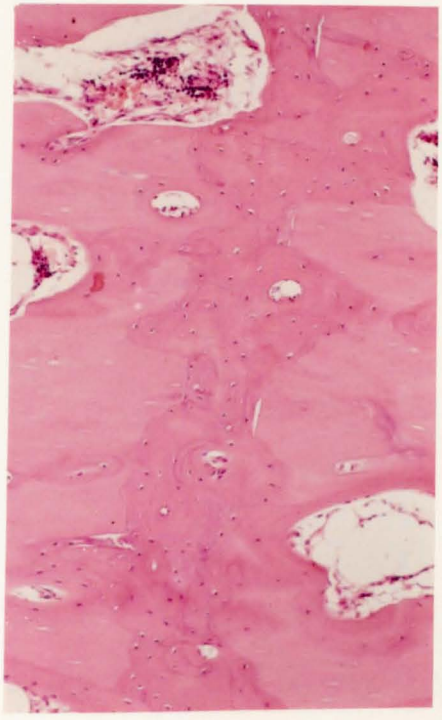
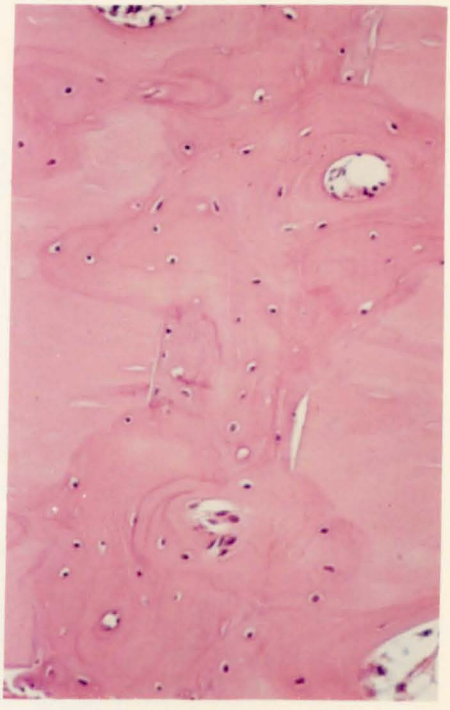

P
nb

FIGURE 3.22 : Longitudinal section of the cortex beneath the plate, at the site of the saw cut. (A) - H and E, magnification x 100. (B) - H and E, magnification x 200. The cut made by the saw can be seen and there has been new bone laid down in the gap. Haversian remodelling has occurred and there is bone bridging the gap.

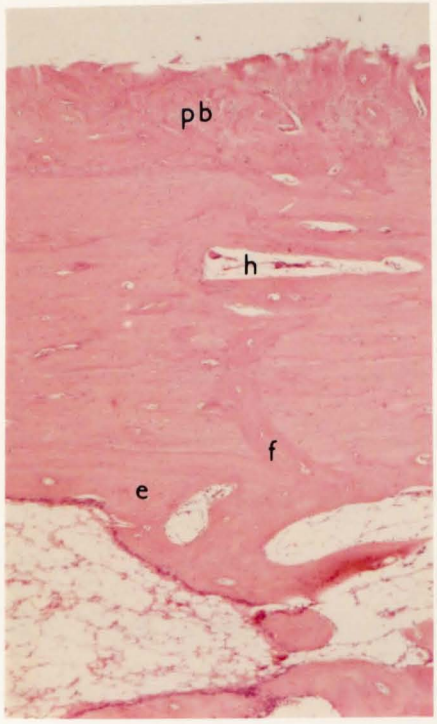


A



B

FIGURE 3.23 : (A) Longitudinal section of the cortex opposite the plate, at the site of the fracture gap. The fracture site (f) can be identified and at the upper end direct bridging by Haversian remodelling has occurred (h). There is some periosteal new bone (pb) and some endosteal ossification (e). H and E. Magnification x 40.
(B) The same section under polarised light,. Although there is some woven bone in the fracture gap inferiorly (w), the appearance at the superior part of the fracture gap suggests that primary bone union has occurred. Magnification x 40.



A

B

group healed by external callus formation. It was often possible to identify the fracture site and the surrounding bridging callus (see Figure 3.24). A marked endosteal reaction was seen around the rod, with new bone formation.

Cortical necrosis was sometimes seen, identified by empty lacunae; it was limited to where the rod was in direct contact with the endosteal surface (see Figure 3.25). Such "contact necrosis" was not a constant finding, being seen in only half the sections where the rod touched the inner cortex. It was variable in depth, but never affected more than 50% of the cortical width. The normal appearance of the cortex was seen where the rod was not in direct contact (see Figure 3.26). These small areas of contact necrosis were equally distributed between proximal and distal tibia.

3.3 Conclusions :

Reproducible midshaft fractures of rabbit tibiae were stabilised with either a dynamic compression plate or a loose-fitting intra-medullary rod. Fractures fixed with a plate ("stable" group) healed with radiological and histological features of primary bone union. In contrast, fractures fixed with an intra-medullary rod ("unstable" group) healed with the typical

FIGURE 3.24 : Longitudinal section across a fracture stabilised with an intramedullary rod. There is bridging callus (c) with new bone (b) in the fracture gap. The original fracture ends are still avascular (a). H and E. Magnification x 40.

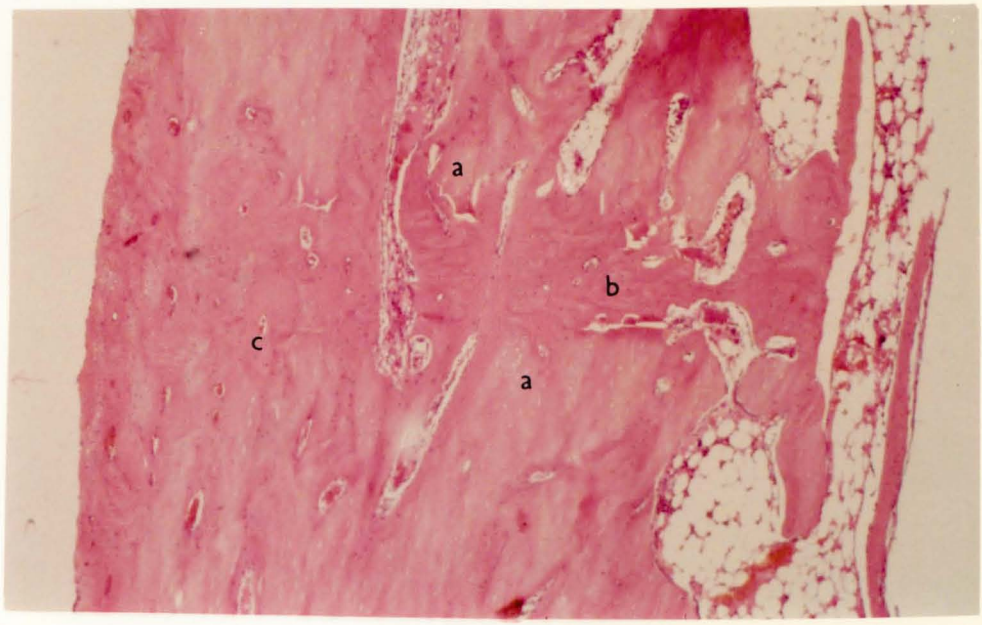

a
b
c
a

FIGURE 3.25 : Transverse section of cortex adjacent to an intramedullary rod. There is avascular necrosis (avn) where the rod (r) has been in contact. H and E. Magnification x 100.

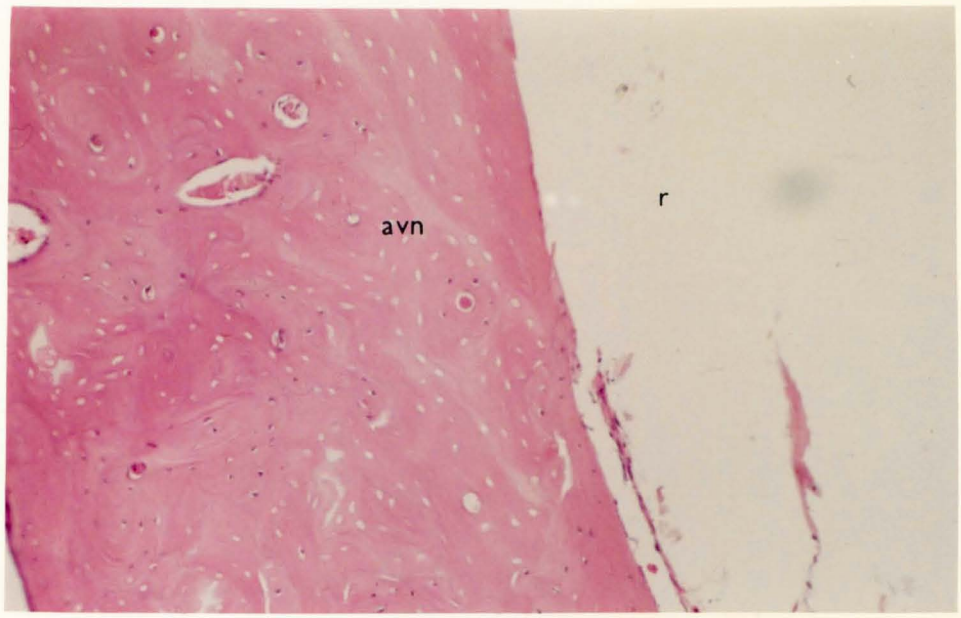
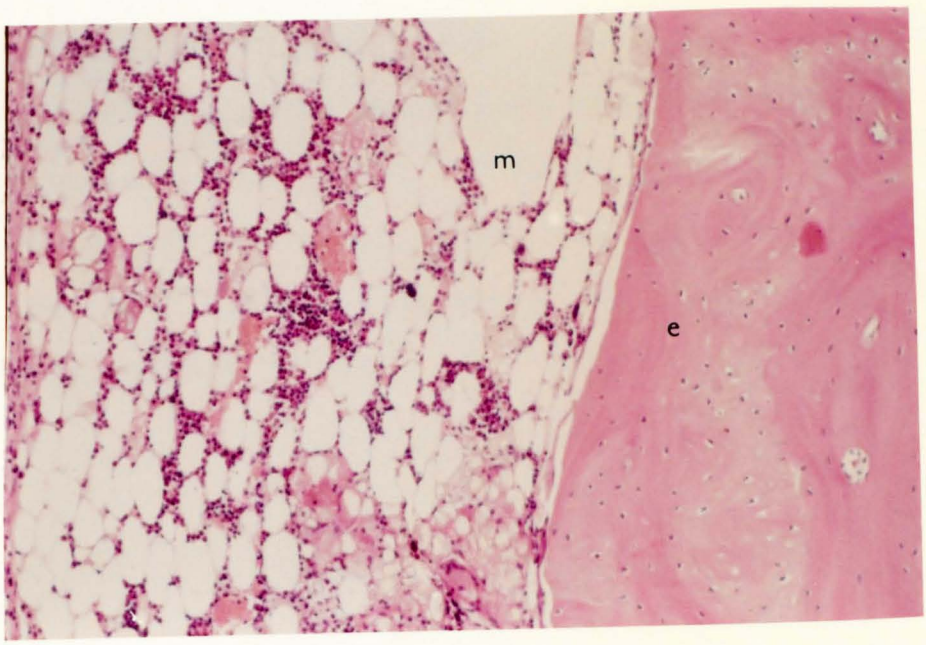

avn
r

FIGURE 3.26 : Longitudinal section to show the endosteal cortex (e) in a fracture fixed with an intramedullary rod. There is no avascular necrosis of the bone; and the gap between the rod and the bone is filled with vascular marrow (m). H and E. Magnification x 100.

features of external callus formation. There were no cases of infection.

Small areas of cortical bone necrosis were seen in relation to both plates and rods. Such necrosis only occurred where the implant was in direct contact with bone. It never affected more than 50% of the cortical width and was seen in less than half the sections where an implant was in direct contact.


m
e

CHAPTER 4

FIXATION OF EXPERIMENTAL FRACTURES WITH DYNAMIC COMPRESSION PLATES AND INTRAMEDULLARY RODS : A MECHANICAL COMPARISON OF THE STABILITY OF THE TWO SYSTEMS

4.1 Methods :

Hicks (1969) defined rigidity of internal fixation devices in terms of the bending moment required to produce angulation at the fracture site. This concept was used by Mason and Fyfe (1979) to compare the rigidity of various techniques of internal fixation in experimental osteotomies of human phalanges.

Mason and Fyfe (1979) produced a standard osteotomy of fresh cadaveric phalanges, which were then fixed with various devices. The phalanges were subjected to load applied to the head of the phalanx and dorsal displacement was measured using a displacement transducer. Load was represented as a bending moment around the fracture site and the displacement as an angular deflection:

a) Bending moment = Load x (fracture to transducer distance)

b) Angular deflection = $\tan^{-1}$ x (displacement ÷ fracture to transducer distance)

The equipment used in my experiment was based on Mason and Fyfe's design (1979), but was modified to allow direct measurement of the angular deformity at the fracture site.

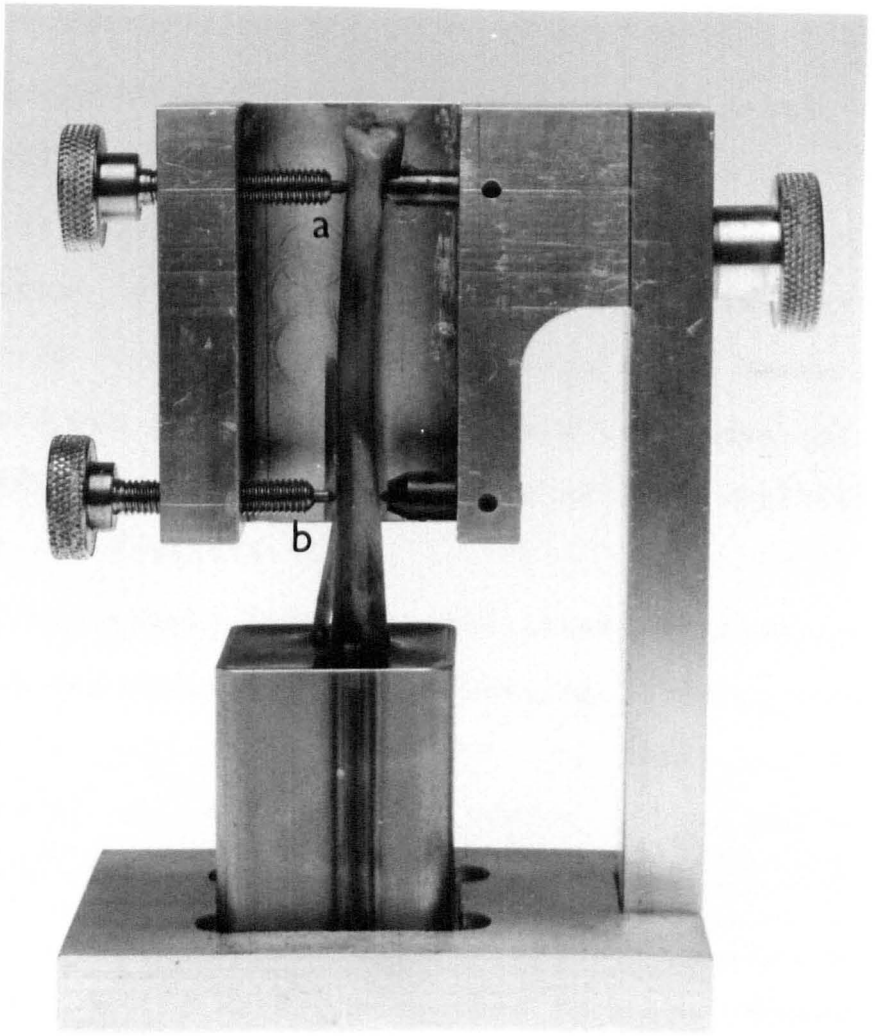
Bone preparation : Rabbit tibiae were obtained from animals sacrificed in the other experiments. The normal tibia was carefully removed in one piece, cleaned of all soft tissue and wrapped in a swab soaked in saline. The tibia was then sealed in a cellophane bag and deep frozen at -25°C until required.

After thawing, the bones were randomly allocated to one of two groups : in eight tibiae a midshaft fracture was produced and fixed with a dynamic compression plate, while in a further eight tibiae the fracture was fixed with an intramedullary rod. The method used in both cases was exactly as described in Chapter 3.1.

The bones were then mounted in a steel container; rigid fixation of the proximal tibia was achieved by mounting the bone in a polyester resin. This resin had been previously reported as capable of resisting loads considerably higher than required for the experiment (Mason and Fyfe 1979).

A special jig was used (see Figure 4.1) to standardise the position of the fracture in relation to the point of application of the load; and to standardise the position of the bone within the polyester resin. Before each bone was fractured it was mounted in the jig. The upper screw (a) was tightened and this marked the point of load application. The lower screw (b) is

FIGURE 4.1 : The mounting jig : This was used to standardise both the position of the fracture and the position of the bone in the resin. The upper screw (a) marks the position of the application of the load. The fracture is created 5mm above the lower screw (b).


a
b

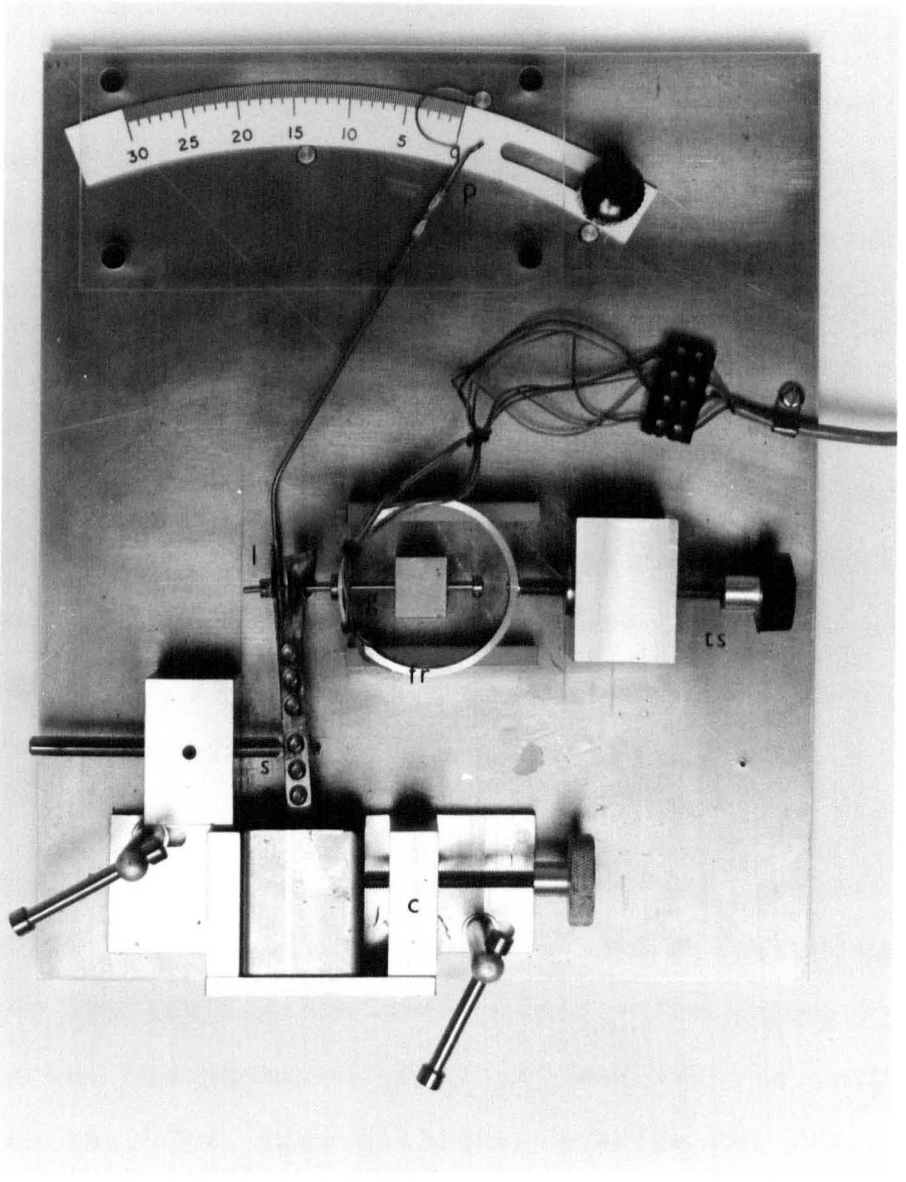
exactly 50 millimetres from the upper screw; when tightened, this lower screw marks the point at which the proximal fragment was supported to prevent elastic deformation. The fracture was always created five millimetres above screw (b). There was therefore a standard distance of 45 millimetres between the point of load application and the fracture.

After fracture and stabilisation, each bone was again mounted in the jig, taking care to ensure that screws (a) and (b) were lined up exactly on the previous marks. The polyester resin was then poured into the steel container and allowed to harden. The bones were kept moist until testing by wrapping them in swabs soaked in saline. An intact tibia is shown mounted in the resin in Figure 4.2.

Recording equipment : The equipment used is shown in Figure 4.3. A load was applied to the distal tibia and the angular deformity measured. The force ring transducer (fr) consisted of an aluminium alloy ring on which were cemented four foil strain gauges. On distortion of the ring, two gauges were in tension and two in compression. The maximum change in reference voltage was obtained using a Wheatstone Bridge arrangement, with strain gauges under similar stress in opposite arms. The voltage obtained was amplified

FIGURE 4.2 : An intact tibia mounted in polyester resin in the steel container.

FIGURE 4.3 : The mechanical testing equipment.
(p) - protractor, (l) - lever arm,
(g) - guide rod, (fr) - force ring transducer,
(ts) - threaded screw, (s) - support rod,
(c) - clamp.


30
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using an integrated circuit differential amplifier, which was calibrated together with the force ring transducer to allow a direct digital reading of the load applied in kilograms.

The angular deformity at the fracture site was measured directly using the lever arm (l) and protractor (p). The lever arm was held lightly against the distal tibia (opposite the point of load application) by a small spring. The angulation at the fracture site was then directly read on the protractor.

Recording procedure : The steel container containing the tibia mounted in resin was clamped into the testing equipment (c). The support rod (s) was the advanced until it was just in contact with the bone, five millimetres below the fracture site. The rod was held in place by the tightening of an Allen screw.

Load was applied to the distal tibia via a flat-ended guide rod (g) by turning the threaded screw (ts). The standard experimental procedure was to increase the load in increments of 0.5 kilograms at 20 second intervals until failure of the bone-fixation complex occurred. The angular deformity at the fracture site was recorded with increasing load; and the load at which failure occurred was noted.

4.2 Results :

Eight fractures were fixed with dynamic compression plates and the graph of the angular deformity at the fracture site in relation to the load applied is shown in Figure 4.4. The mean load to failure of all eight specimens was 5.4 kilograms (SD ± 2.0). Specimen five withstood a much greater load before failing than the other tibiae, which all failed at loads between 4.0 kilograms and 6.0 kilograms.

Eight fractures were fixed with intra-medullary rods and the graph of the angular deformity at the fracture site in relation to the load applied is shown in Figure 4.5. The mean load to failure of all eight specimens was 6.4 kilograms (SD ± 1.68). Specimen four failed at a much higher load than the other tibiae, with a greater angular deformity before failure. There was no statistically significant difference in the mean load to failure between fractures fixed with compression plates and fractures fixed with intramedullary rods.

The bending moment at the fracture site was calculated up to a load of five kilograms from the equation:

Bending moment = Load x (fracture to transducer distance)

The mean angular deformity for a given bending

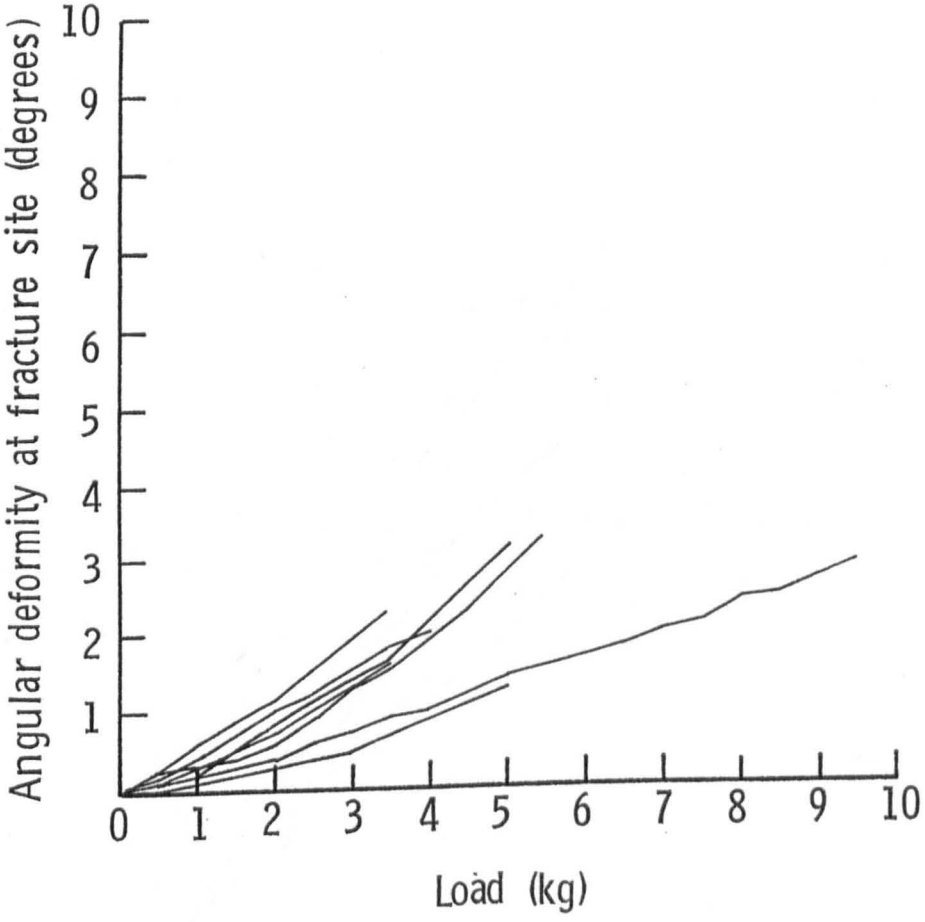


FIGURE 4.4 : Fractures fixed with dynamic compression plates : Graph of the angular deformity after load application. All eight specimens were tested until the fixation failed.

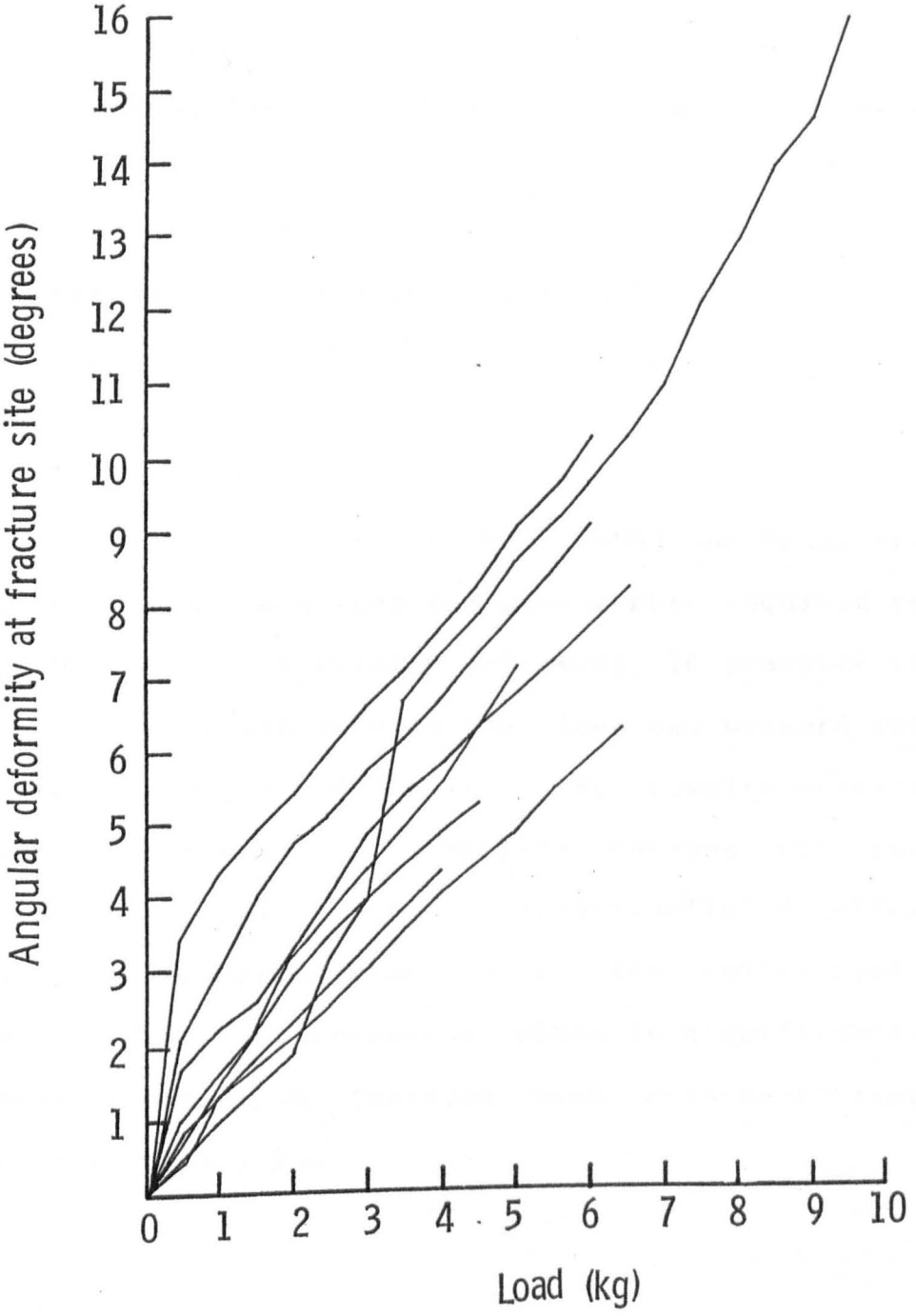


FIGURE 4.5 : Fractures fixed with intramedullary rods : The angular deformity after load application. All eight specimens were tested until the fixation failed.

moment was also calculated for both the "plate fixed" group and the "rod-fixed" group. The results are tabulated in Table 4.1. Fractures fixed with an intramedullary rod deform more for a given bending moment than fractures fixed with a compression plate. These differences in mean angulation were statistically significant throughout the measured range. The results are expressed graphically in Figure 4.6.

4.3 Conclusions

Although both Hicks (1969) and Mason and Fyfe (1979) measured the mean moment required to produce a given angular deformity, in practice it is easier to standardise the load and measure the resulting angular deformity. My results confirm the difference in stability between the two fixation systems tested. Mechanically, fixation of an experimental fracture of the rabbit tibia with a dynamic compression plate is significantly more rigid than fixation with a loose-fitting intramedullary rod.

Table 4.1 : Mean angular deformity at the fracture site for a given load in fractures fixed with both plates and intramedullary rods. (Nm = Newton metres, t = Student's t test, * $0.001<p<0.01$, ** $p<0.001$)

	Mean angular deformity at fracture site		t	DF	
	Rods	Plates			
Bending moment (Nm)					
2.2	1.3° ±1.1°	0.1° ±0.07°	3.252	14	*
4.4	2.0° ±1.1°	0.3° ±0.1°	4.375	14	**
6.6	2.6° ±1.2°	0.5° ±0.2°	4.811	14	**
8.8	3.2° ±1.3°	0.7° ±0.3°	5.558	14	**
11.0	3.8° ±1.2°	1.0° ±0.3°	6.609	14	**
13.2	4.4° ±1.2°	1.2° ±0.4°	6.972	14	**
15.5	5.2° ±1.3°	1.5° ±0.5°	5.854	14	**
17.7	5.8° ±1.4°	1.6° ±0.6°	6.313	11	**
19.9	6.5° ±1.4°	1.8° ±0.8°	6.031	9	**
22.1	7.4° ±1.5°	2.2° ±1.0°	5.973	8	**

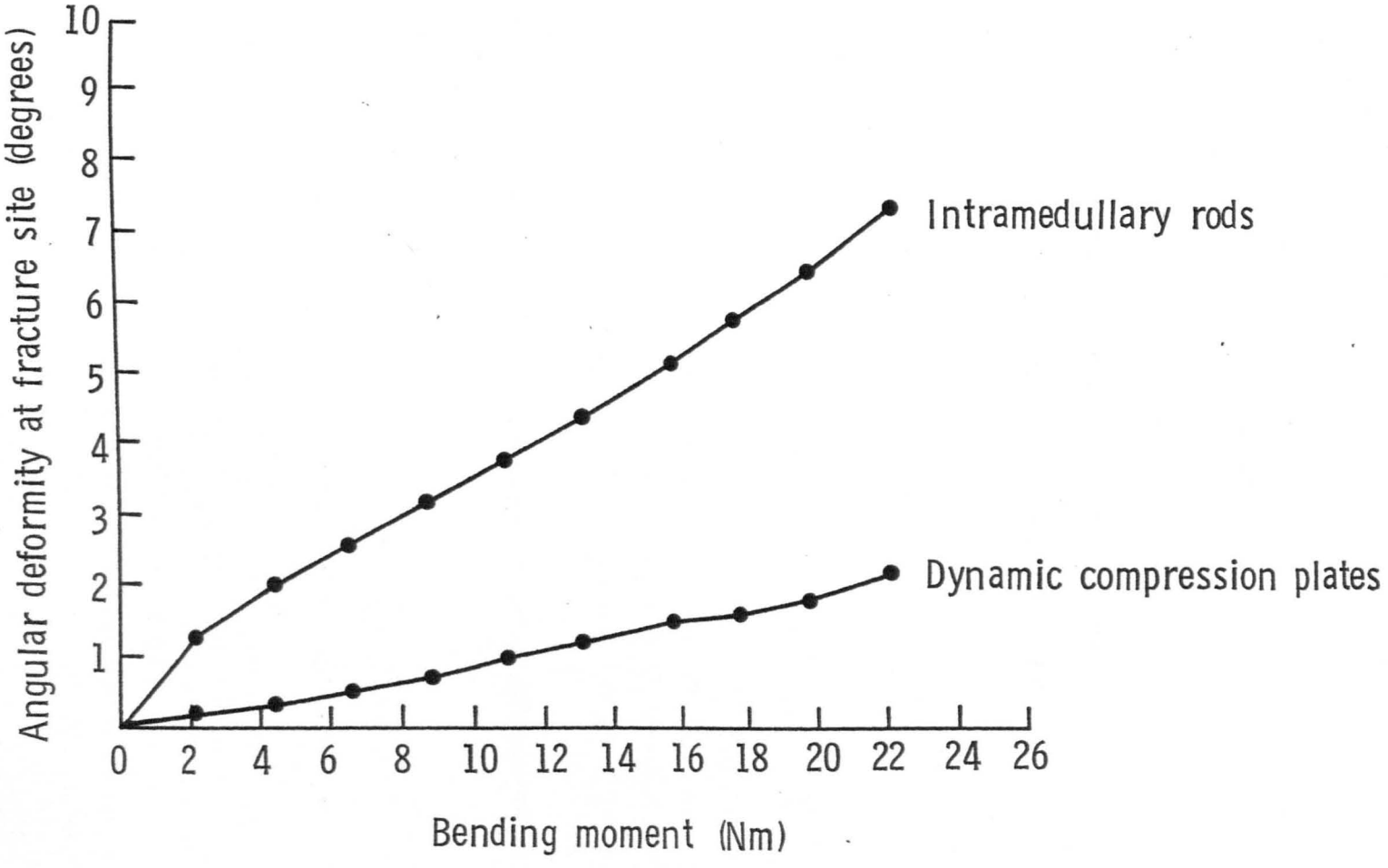


FIGURE 4.6 : Graph of the mean angular deformity at the fracture site in relation to the bending moment applied.

CHAPTER 5

AN EXPERIMENTAL MODEL OF POST-TRAUMATIC OSTEOMYELITIS

5.1 Methods :

The rod-fixed fracture model (as described in Chapter 3) was used in my pilot study. The aim was to identify an appropriate strain of Staphylococcus aureus, which would reliably induce osteomyelitis under these experimental conditions. It was also necessary to determine the minimum inoculum size for infection; and to define the characteristic features of the osteomyelitis, both radiologically and histologically.

Twenty-three rabbits were used in the pilot study. In each rabbit, a midshaft fracture of the tibia was produced as previously described and stabilised with a loose-fitting intramedullary rod (see Chapter 3.1).

The bacteria were inoculated directly into the fracture site, after skin closure, through a 23G needle. Four different strains of Staphylococcus aureus were initially assessed:

1) COM 1 : As stated before (Chapter 2.3) this strain was isolated from a patient with chronic osteomyelitis. It was of phage type 29 and its characteristics have been reported by Coleman et al (1983). Three inoculum sizes were used: 10^5, 10^6 and 10^7 organisms.

2) Strain "B" : An isolate from a patient with sub-acute bacterial endocarditis. It was of phage

type 96 It was used solely in an inoculum of 10^6 organisms.

3) Strain "G" : An isolate from a child with acute haematogenous osteomyelitis. It was of phage type 54/85. It was used solely in an inoculum of 10^6 organisms.

4) Strain "W" : A nasal isolate from a human asymptomatic carrier. It was only used in an inoculum of 10^6 organisms.

Organisms were prepared as described in Chapter 2.3 and were diluted in buffered phosphate saline to give the required inoculum in 0.5ml. Inoculation was performed by drawing up 0.5ml of the bacterial suspension into a 1.0ml tuberculin syringe and injecting it into the fracture site via the 23G needle. The needle, containing the remaining inoculum, was then flushed with 0.1ml of 0.9% saline solution, using another syringe. This ensured that the full inoculum was injected into the fracture site. The 23G needle was then withdrawn.

The post-operative management for all animals was as described in Chapters 2.2 and 3.1. All rabbits were assessed using the standard protocols (Chapter 2.5) and were sacrificed 12 weeks after-operation, using the technique given in Chapter 2.4.

5.2 Results :

Initially a group of five animals were inoculated with the COM 1 organism in a concentration of 10^6 organisms. Three groups, with three rabbits in each group, were used to assess the other three strains of Staphylococcus aureus. Finally, the effect of inoculum size was studied using the COM 1 organism; four rabbits were inoculated with 10^5 organisms and five rabbits with 10^7 organisms. No technical problems were encountered and there were no deaths. All 23 animals survived the experimental protocol.

Clinical assessment :

In all rabbits the wound became swollen and erythematous within 24 hours of operation. This inflammation persisted for a minimum of two weeks and resolved within four weeks in 15 animals. Obvious fracture callus was palpable until six to eight weeks after operation. Clinically, these 15 animals showed no sign of infection.

However, in the other eight rabbits the lower leg which had been operated on remained markedly swollen throughout the 12 weeks. In three of these rabbits, an abscess formed medially, at the level of the fracture site. These animals had all been inoculated with the COM 1 strain in a concentration of 10^7 organisms. The abscesses all drained pus spontaneously, after

which the skin healed. Persistent sinus formation did not occur. These eight rabbits were graded as clinically infected.

In two animals a small, superficial sore developed over the medial malleolus. These sores healed quickly, without any noticeable discharge; and were considered to have been caused by the rabbit nibbling at the skin.

At sacrifice, in the animals clinically thought to be normal, there was no macroscopic sign of infection. There was minimal scarring and the periosteum was not thickened or inflammed. On division of the distal tibia to obtain a culture swab from around the implant, a normal appearance was seen.

In contrast, in the clinically infected animals there were marked vascular adhesions around the tibial shaft. The periosteum was thickened, as was the tibial shaft distally. No obvious soft tissue abscesses were seen, but on division of the distal tibia there was always a variable amount of thick, white pus around the implant. Occasionally, an obvious bony sequestrum was found in the pus.

Microbiological assessment :

No organisms were isolated from the culture swabs taken from around the implants in the 15 rabbits which were considered clinically not to be infected (hereafter termed non-infected

rabbits). Staphylococcus aureus (of phage type 29) was isolated from the discharge of all three clinical abscesses. All eight of the clinically infected rabbits had positive cultures of Staphylococcus aureus (phage type 29) from swabs taken from around the implants.

Radiological assessment :

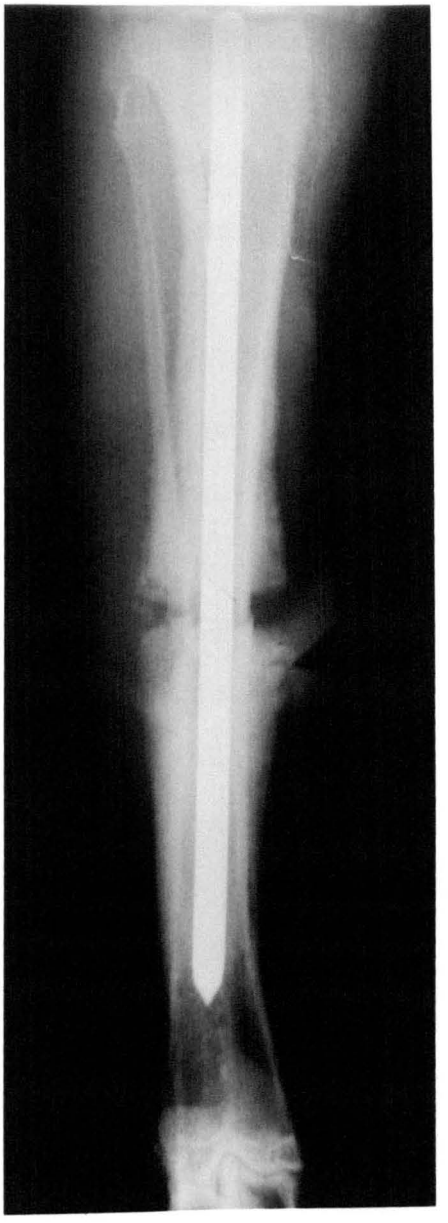
There was good conformity between the candidate and the radiologist on assessment of the radiographs. There was complete agreement both on the diagnosis of osteomyelitis and the pattern of bone union.

Healing by external callus formation was seen in the non-infected rabbits. This was identical in pattern to that seen in the control animals (see Chapter 3), except that soft-tissue swelling was always seen on the radiographs obtained after two weeks. This swelling persisted until four weeks in three rabbits.

In contrast, a distinct and characteristic radiological pattern was seen in the eight clinically infected rabbits. The fracture line remained clearly visible in all animals at four weeks; and in two animals it did not disappear until after six weeks.

A "fluffy" periosteal reaction appeared at two weeks (see Figure 5.1); this never involved the fracture site itself, but began some three to four millimetres away from the fracture. This

FIGURE 5.1 : Radiograph obtained two weeks after fixation with an intramedullary rod and inoculation with bacteria. There is obvious periosteal reaction, but not at the fracture site.



periosteal reaction increased in extent and reached its maximum at four to six weeks, persisting thereafter until sacrifice. The periosteal reaction invariably extended distally, rather than proximally. It was always followed by the appearance of an involucrum at the four to six week stage (see Figure 5.2).

Callus was not usually exuberant and merged imperceptibly with the periosteal reaction. In all eight rabbits bony union occurred by bridging external callus. Sclerosis and osteolysis were late features of infection, appearing at six to eight weeks or later. Their appearance was variable : six animals showed some evidence of sclerosis; and osteolysis around the implant, usually with some cortical erosion, was seen in six rabbits (see Figure 5.3). The characteristic radiological features of bone infection can be seen on serial radiographs obtained from an infected animal (see Figure 5.4.).

Histological assessment :

In the fifteen non-infected rabbits, the pattern of bone union was the same as seen in the control group (see Chapter 3). There were no signs of infection in these animals.

The remaining eight rabbits (clinically and radiologically infected) showed characteristic changes of chronic osteomyelitis. There was a

FIGURE 5.2 : Radiograph obtained six weeks after fixation with an intramedullary rod and inoculation with bacteria. There is the radiological appearance of an involucrum.

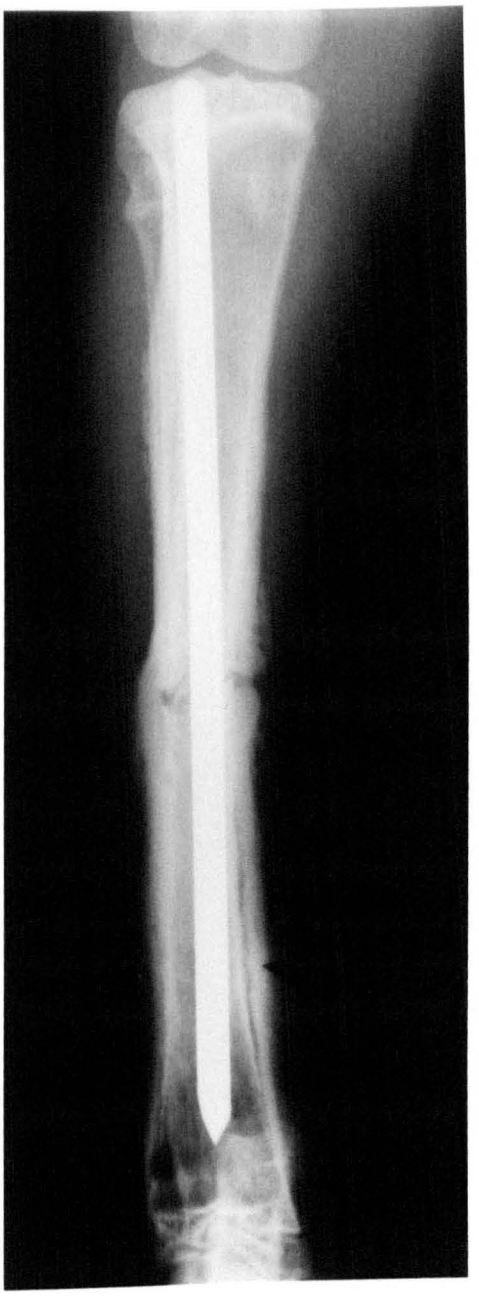


FIGURE 5.3 : Radiograph obtained ten weeks after fixation with an intramedullary rod and inoculation with bacteria. There is sclerosis close to the fracture and lysis distally, with obvious cortical erosion.

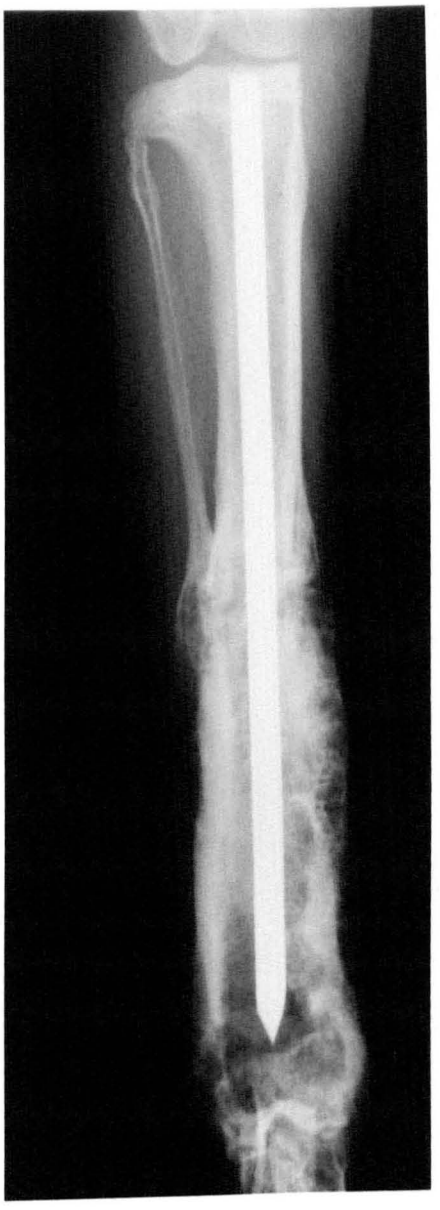
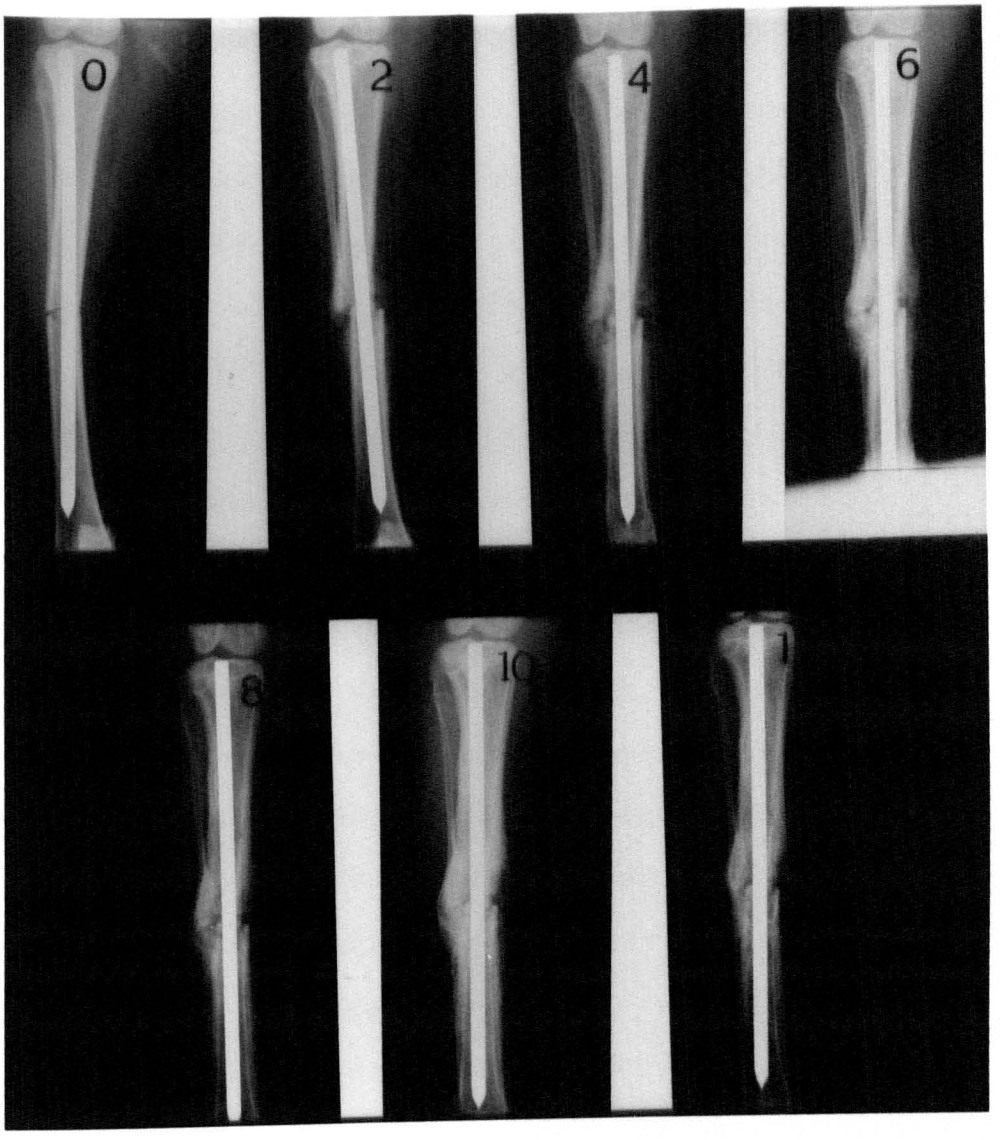


FIGURE 5.4 : Serial radiographs after fixation with an intramedullary rod and inoculation with bacteria, obtained at two-weekly intervals. The typical radiological appearances of osteomyelitis are seen : periosteal reaction, involucrum formation and sclerosis.


0
2
4
6
8
10
1

marked polymorphonuclear leucocyte reaction with abscess formation around the rod track (see Figure 5.5). In several animals the site of the original fracture could be identified. There was necrosis of cortical bone with abscess formation (see Figure 5.6).

There had been marked sub-periosteal new bone formation with necrosis of the original cortex. Sequestra were always seen : occasionally small pieces of dead bone with bacteria visible within the Haversian systems were observed (see Figure 5.7). More commonly seen was a large sequestrum encased by an involucrum (see Figure 5.8). This corresponds to the typical radiographic appearance seen in Figure 5.2. Gram-positive cocci were frequently identifiable on the corresponding sections stained by Gram's method (see Figure 5.9).

5.3 Conclusions :

The results are summarised in Table 5.1. Apart from strain "W", all the Staphylococci were capable of inducing infection in an inoculum of 10^6 organisms. There was a slightly higher rate of infection with the COM 1 strain and, as its characteristics were well defined (Coleman et al, 1983), this strain was chosen for the remainder of the study.

This organism was then used to assess the

FIGURE 5.5 : Transverse section across an infected rod-track (r). There are small sequestra and debris (d) around the rod, with marked polymorphonuclear leucocyte (pnl) reaction. H and E. Magnification x 40.

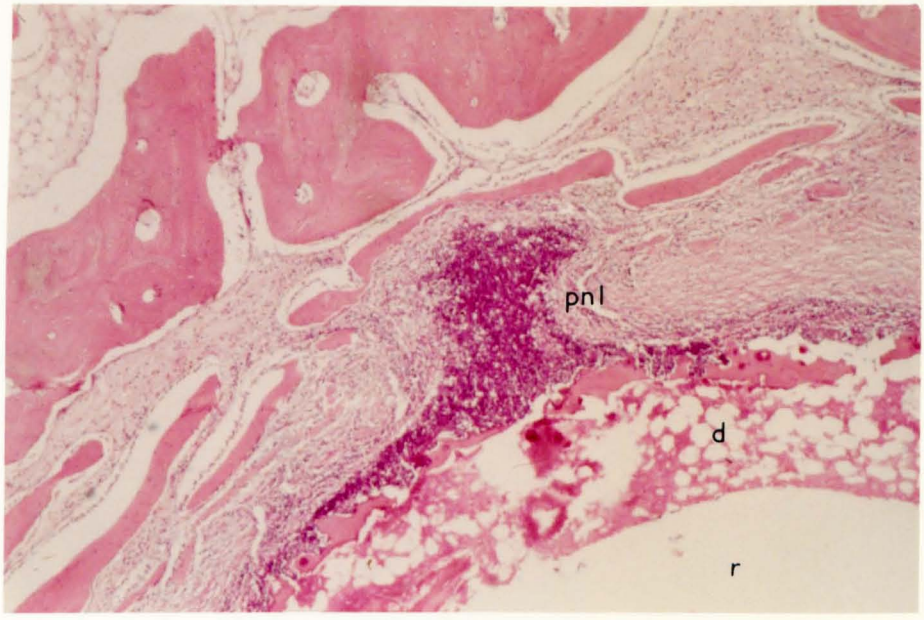

pnl
d
r

FIGURE 5.6 : The cortical bone at the site of the saw cut has formed a sequestrum (s). There is an abscess (a) surrounding this sequestrum. There is obvious sub-periosteal new bone (pb) formation. H and E. Magnification x 40.

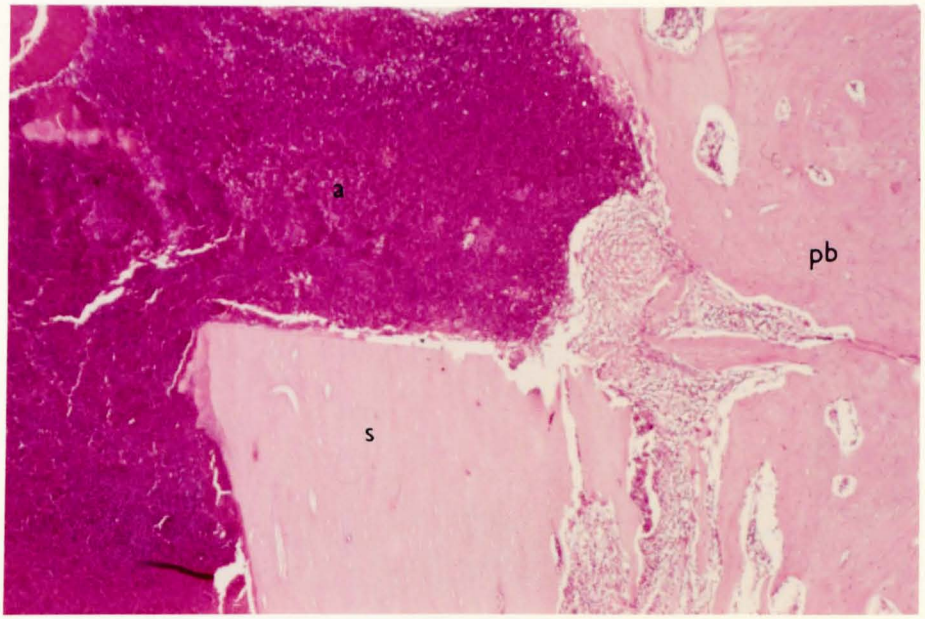

a
pb
s

FIGURE 5.7 : A sequestrum (s) containing clumps of bacteria (b), surrounded by a marked polymorphonuclear leucocyte (pnl) reaction. H and E. Magnification x 100.

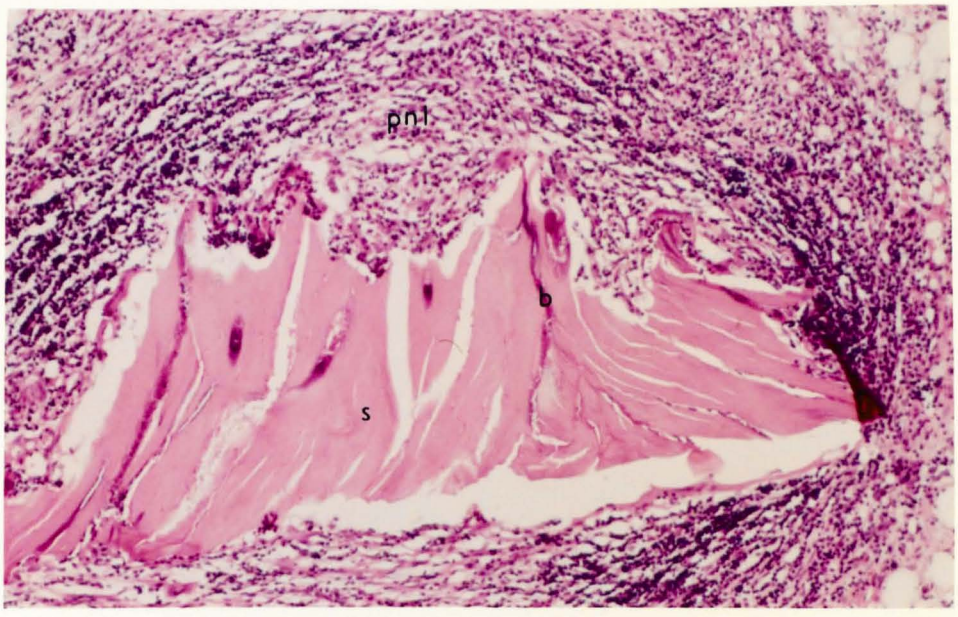

pnl
b
s

FIGURE 5.8 : A sequestrum (s) surrounds the rod track (r); and an involucrum (i) encases the sequestrum. H and E. Magnification x 40.

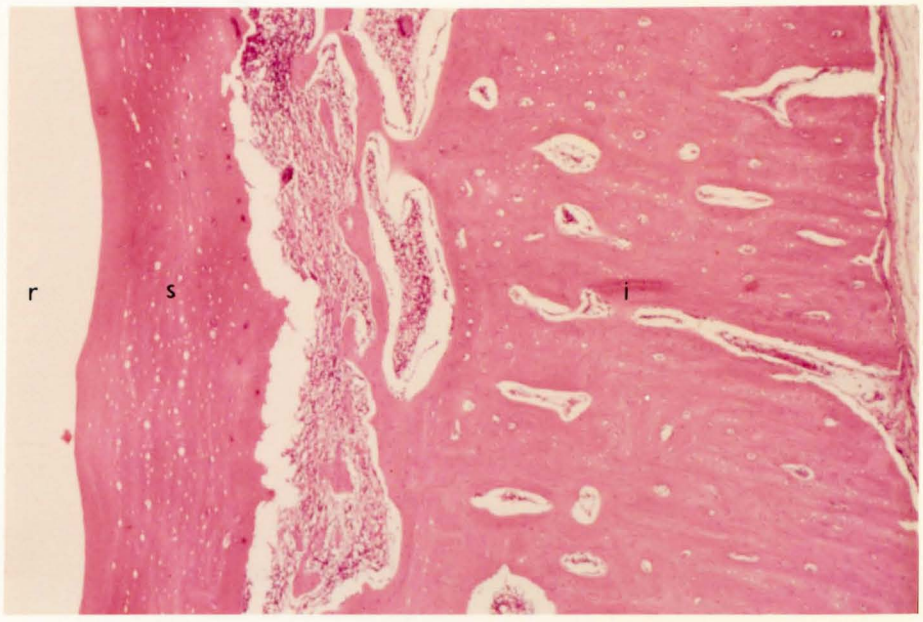

r
s
i

FIGURE 5.9 : The same section as in Figure 5.7, stained by Gram's method. Clumps of gram-positive bacteria (b) are seen in the sequestrum (s). There is surrounding polymorphonuclear leucocyte (pnl) reaction. Magnification x 200.

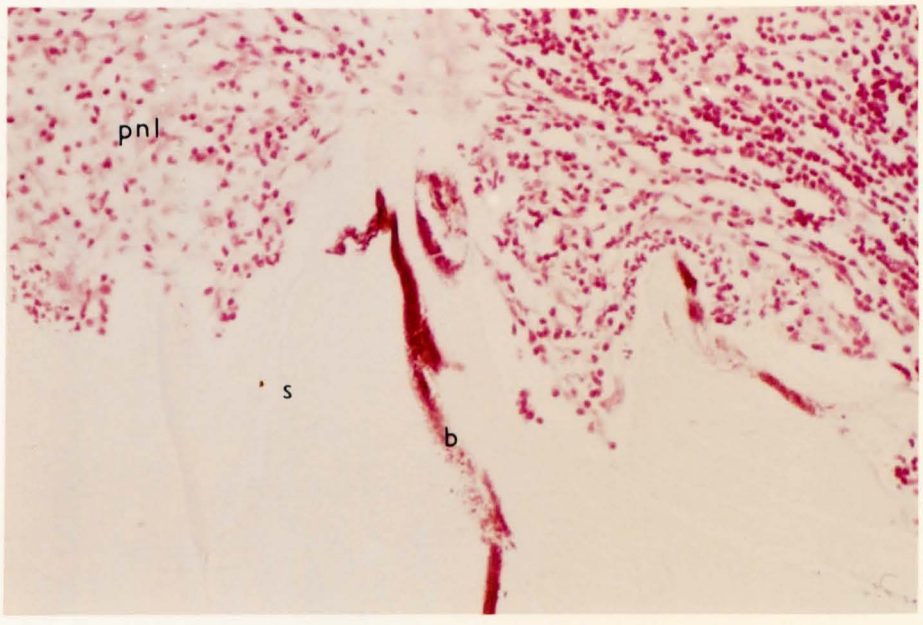

pnl
s
b

TABLE 5.1 : Distribution of animals by strain of organism and inoculum size

ORGANISM	INOCULUM	NO OF RABBITS IN GROUP	NO OF INFECTIONS IN GROUP
"W"	10^6	3	0
"G"	10^6	3	1
"B"	10^6	3	1
COM 1	10^5	4	0
COM 1	10^6	5	2
COM 1	10^7	5	4
		23 Rabbits	8 Rabbits

effect of differing sizes of inoculum. No cases of infection were seen with an inoculum of 10^5 organisms; when this was increased to 10^7 organisms, infection occurred in 80% of the animals.

There was a correlation between each of the clinical, radiological, microbiological and histological methods for assessing infection. However, the clinical signs were more variable and for the remaining phases of the study radiological, microbiological and histological criteria were used to diagnose infection.

CHAPTER 6

PREVENTION OF INFECTION IN EXPERIMENTAL OPEN FRACTURES : THE EFFECT OF FRACTURE STABILITY

6.1 Methods :

To assess the effect of stability in preventing bone infection, it was necessary to perform both stable fixation and unstable fixation of experimental open fractures. The mechanical tests reported in Chapter 4 showed that stabilisation of a tibial fracture with a dynamic compression plate is a very rigid form of fixation. The loose-fitting intramedullary rod is unstable both axially and rotationally, although it maintains axial alignment.

The results in the control groups (Chapter 3) confirmed that it is technically possible to produce a fracture and stabilise it with plates or rods. Fractures reduced anatomically and held with dynamic compression plates heal by primary bone union, whilst fractures stabilised with intramedullary rods heal by external callus formation. There were no cases of infection in the control animals.

The pilot study of experimental osteomyelitis (Chapter 5) showed that inoculation with the COM 1 organism, in concentrations of both 10^6 and 10^7 organisms, would produce osteomyelitis. The radiological, microbiological and histological features of this post-traumatic

osteomyelitis have been clearly defined.

For the definitive study on the effect of stability, 45 rabbits were used. Animals were randomly allocated to one of two main groups, each of which was divided into two sub-groups:

1) Stable group : Using the techniques described in Chapter 3.1, experimental fractures of the rabbit tibia were reduced and held with a dynamic compression plate. The fracture site was inoculated with the COM 1 organism as described in Chapter 5.1. Two different concentrations of Staphylococci were used: 10^6 and 10^7 organisms, both in 0.5ml buffered phosphate saline.

2) Unstable group : Using the method described in Chapter 3.1, experimental fractures of the rabbit tibia were reduced and held with loose-fitting intramedullary rods. The fracture site was inoculated with the COM 1 organism as described in Chapter 5.1. Two different concentrations of Staphylococci were used: 10^6 and 10^7 organisms, both in 0.5ml buffered phosphate saline.

After withdrawal of the 23G needle and protection of the wound with Nobecutane, the post-operative management of both groups was as described in Chapters 2.2 and 3.1. All rabbits were assessed using the standard protocols (Chapter 2.5) and were sacrificed 12 weeks after operation, using the technique given in Chapter 2.4.

Infection was diagnosed if the following criteria were met:

1) Radiological appearance of periosteal reaction, involucrum or sequestrum formation, osteolysis around the implant and sclerosis.
2) Isolation of Staphylococcus aureus (phage type 29) from the culture swab taken from around the implant at sacrifice.
3) Histological evidence of polymorphonuclear leucocyte reaction, abscess formation, the presence of gram-positive cocci on Gram staining, bone necrosis and sub-periosteal new bone formation.

6.2 Results :

Of the 45 rabbits initially entered into this experiment, 21 were in the unstable group. The fracture site was inoculated with 10^6 Staphylococci in ten animals and with 10^7 Staphylococci in eleven others. There were no complications or deaths and all 21 animals survived the experiment.

However, four rabbits in the stable group have been excluded from analysis. A fracture of the distal tibia occurred early in the first week after operation in two rabbits; they were

immediately sacrificed. In one other rabbit an undisplaced distal tibial fracture occurred at two weeks. The animal was not distressed and was sacrificed as planned at 12 weeks; it was excluded from the study group. In a fourth rabbit the needle tip broke and could not be retrieved and the animal was excluded. The presence of this metallic foreign body caused no obvious problem and the rabbit was sacrificed as planned at 12 weeks. There were, therefore, a total of 20 rabbits in the stable group: the fracture site was inoculated with 10^6 Staphylococci in ten animals and with 10^7 Staphylococci in the other ten rabbits.

The results are summarised in Table 6.1 and will be further considered below.

Unstable Group :

Infection was confirmed in five of the ten rabbits inoculated with 10^6 organisms and in ten of the eleven inoculated with 10^7 organisms. There was no difference in the severity of infection between these two groups. There was a correlation between each of the clinical, radiological, microbiological and histological criteria for diagnosing infection.

There were no obvious differences in the findings on assessment between these animals and those of the pilot study (Chapter 5), in which the

TABLE 6.1 : Osteomyelitis rate in relation to fracture stability

GROUP	NO OF RABBITS IN GROUP	NO OF INFECTIONS IN GROUP*
"Stable" (Dynamic Compression Plate)	20	7
"Unstable" (Loose Intra-medullary Rod)	21	15
	41 rabbits	

*x^2 = 5.47, DF=1, 0.01<p<0.02

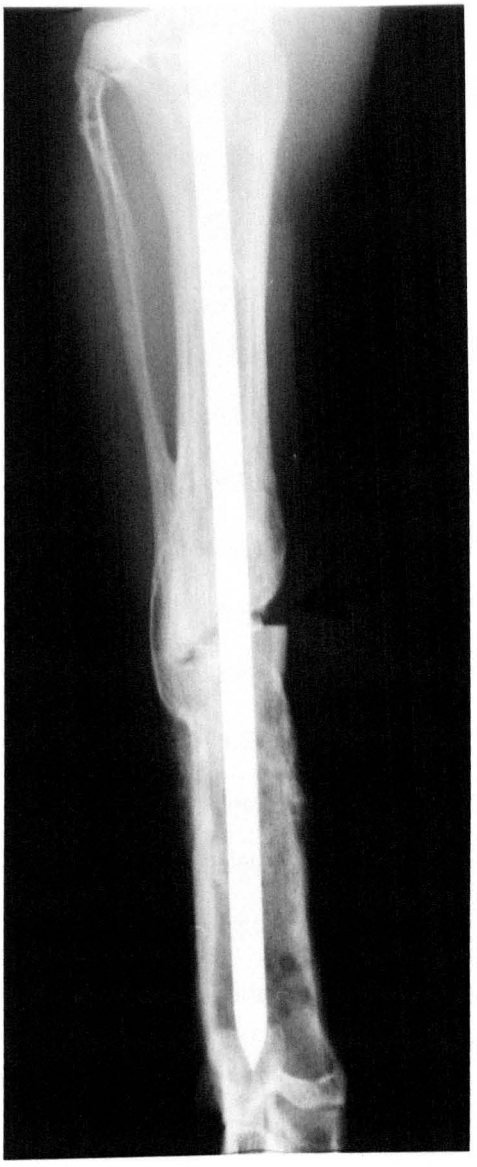
fracture was stabilised with an intramedullary rod. In those animals graded as "non-infected", the swelling and erythema around the wound settled within two weeks. Serial radiographs showed uneventful healing by external callus formation. No organisms were isolated from around the rod at sacrifice and no histological evidence of infection was seen.

The 15 infected animals had the characteristic diffuse swelling of the leg throughout the 12 weeks and frank abscess formation occurred in four rabbits. All these animals had been inoculated with 10^7 organisms; and in one a persisting discharging sinus developed.

The serial radiographs all showed the typical pattern of persistence of the fracture line for at least four to six weeks, the development of an involucrum at around six weeks and the later appearance of sclerosis and osteolysis (at six to eight weeks). Bony bridging by external callus formation was seen in all 15 rabbits, but in one it was tenuous (see Figure 6.1).

Staphylococcus aureus (of phage type 29) was isolated in all 15 rabbits from around the rod at sacrifice. The typical histological signs of infection were seen in all : polymorphonuclear leucocyte reaction, abscess formation,

FIGURE 6.1 : Radiograph obtained twelve weeks after fixation with an intramedullary rod and inoculation with bacteria. There is bridging callus laterally, but the fracture site is still obvious medially.



sub-periosteal new bone formation and sequestrum formation (see Chapter 5.2).

Stable Group :

Infection was confirmed in three of the ten rabbits inoculated with 10^6 organisms and four of the ten inoculated with 10^7 organisms.

The clinical appearance of the leg was not wholly reliable in diagnosing infection. In ten rabbits (all subsequently confirmed as non-infected), the swelling and erythema settled within a week and the appearance was no different from that seen in the control animals (see Chapter 3). In five animals slight, but definite, swelling of the mid-part of the lower leg persisted until sacrifice; infection was subsequently confirmed in only three of these animals. The remaining five rabbits had generalised diffuse swelling of the whole lower leg; infection was subsequently confirmed in all of these animals.

The macroscopic appearance of the tibia at sacrifice was much more reliable. Thickened periosteum and dense vascular adhesions were seen in seven rabbits; in five there was an obvious abscess adjacent to the plate. These appearances were similar to those seen in the infected fractures fixed with rods (see Chapter 5.2). In the remaining 13 animals, the macroscopic

appearance was identical to that seen in the control animals (Chapter 3.2) - namely, a normal-looking tibia, no adhesions and minimal new bone formation along the plate.

A positive culture of Staphylococcus aureus (phage type 29) was seen in the four rabbits, inoculated with 10^7 organisms and in which there was macroscopic infection. No organisms were identified from the remaining six rabbits in this group which lacked signs of infection. There was only one positive culture from the animals inoculated with 10^6 organisms: this was from the one rabbit in this group with an obvious abscess next to the plate. There were two other animals in this group which were considered to be infected at sacrifice; but no pus was found and no organisms were grown. However, both these rabbits showed the typical radiological and histological appearances of bone infection and have been graded as "infected".

The pattern of fracture healing in rabbits, under conditions of anatomical reduction and stable fixation, was described in Chapter 3. All thirteen rabbits thought to be "non-infected", on the basis of appearance at sacrifice and negative bacteriological culture, showed this typical appearance of healing without external callus formation. In one rabbit, the periosteal reaction around the lateral screw holes was more

marked and persisted until sacrifice (see Figure 6.2).

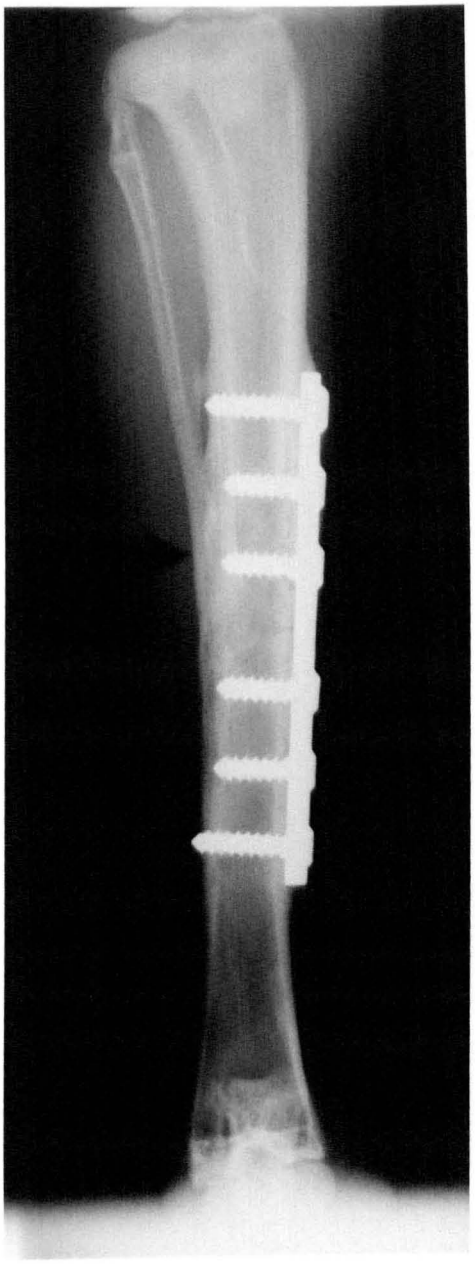
A different radiological appearance was seen in the other seven rabbits. In each animal, "fluffy", immature periosteal reaction was seen after two weeks; and there was obvious soft-tissue swelling on the radiograph in five of the seven animals (see Figure 6.3). This periosteal reaction reached its maximum extent by six weeks, sometimes being very marked. It was invariably immature new bone and limited to around the plate (see Figure 6.4).

Osteolysis around the screws was seen in all the rabbits (see Figure 6.5). This bone loss usually appeared at six to eight weeks, but in one animal it was obvious on the radiograph obtained at four weeks. Sclerosis was only seen in three rabbits; it was a late feature, appearing after eight to ten weeks. There was no radiological evidence of involucrum or sequestrum formation in any rabbit. There was healing by external callus formation in each of these seven animals.

The typical features of bone infection after compression plating are seen in the serial radiographs of one of the infected animals (see Figure 6.6.).

All seven rabbits graded as infected on radiological criteria showed the typical histological features of chronic osteomyelitis.

FIGURE 6.2 : Radiograph obtained twelve weeks after fixation with a dynamic compression plate and inoculation with bacteria. Although there is mature periosteal reaction laterally, there are no other signs of infection and the fracture has united uneventfully.



IGURE 6.3 : Radiograph obtained two weeks after fixation with a dynamic compression plate and inoculation with bacteria. There is marked immature periosteal reaction laterally and the fracture line is visible.

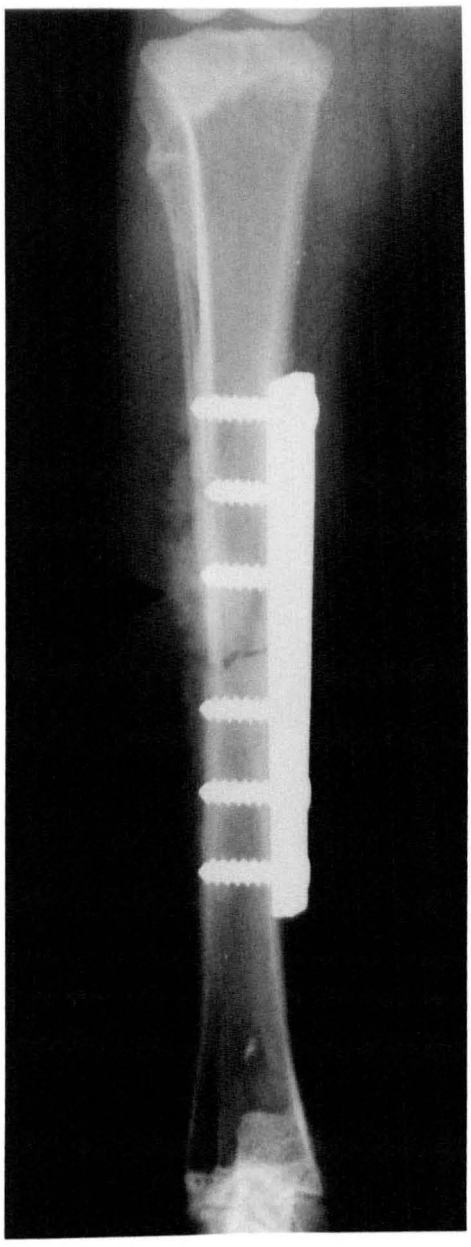


FIGURE 6.4 : Radiograph obtained six weeks after fixation with a dynamic compressin plate and inoculation with bacteria. This shows extensive periosteal reaction, limited to around the plate. The fracture site is still visible.

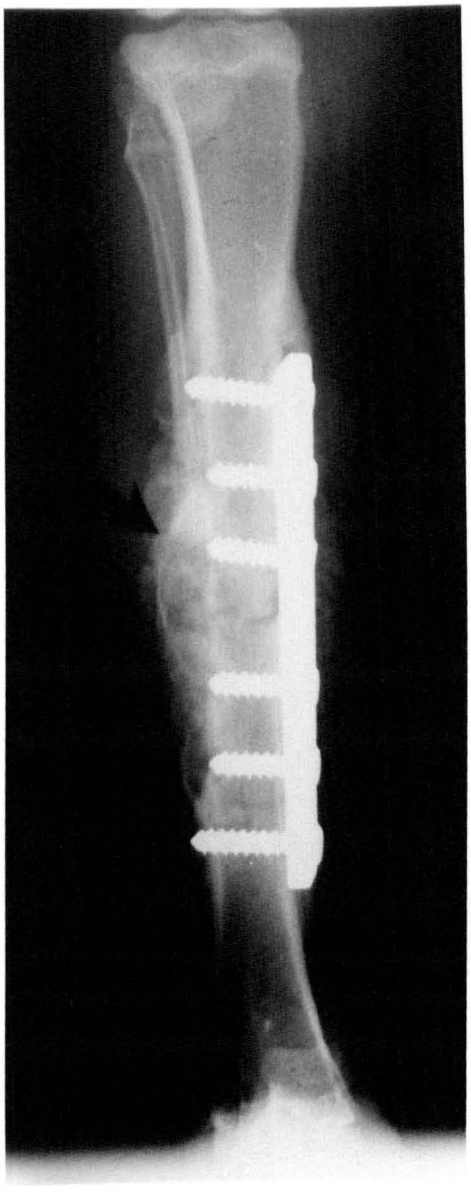


FIGURE 6.5 : There is osteolysis around the distal screws in this radiograph, obtained eight weeks after fracture and inoculation with bacteria.

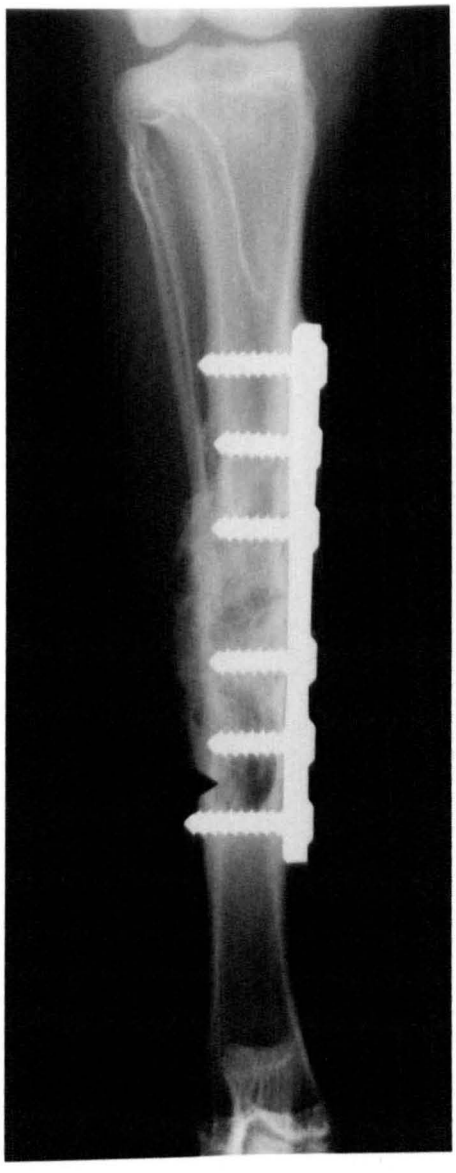
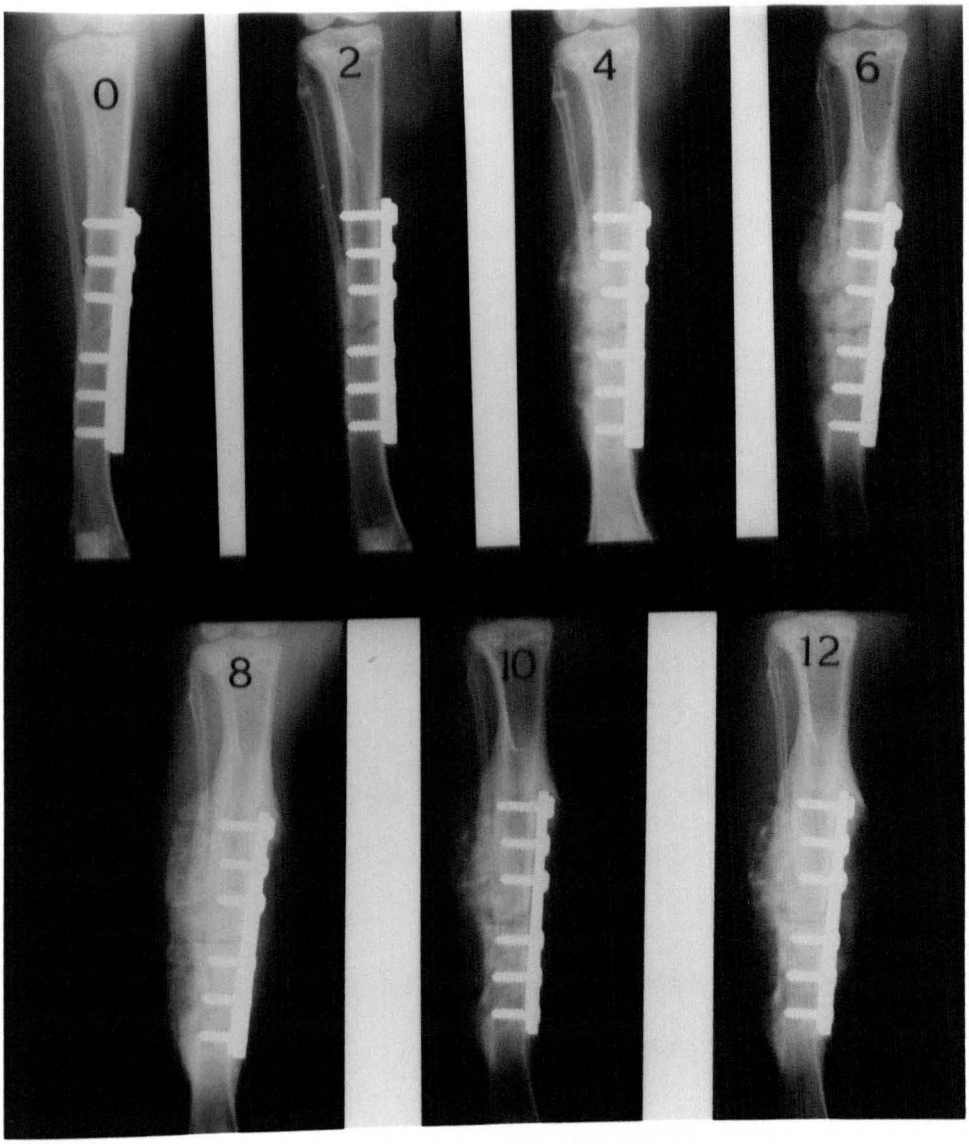


FIGURE 6.6 : Serial radiographs after fixation with a dynamic compression plate and inoculation with bacteria, obtained at two weekly intervals. This shows the typical radiological features of infection around a plate.


0
2
4
6
8
10
12

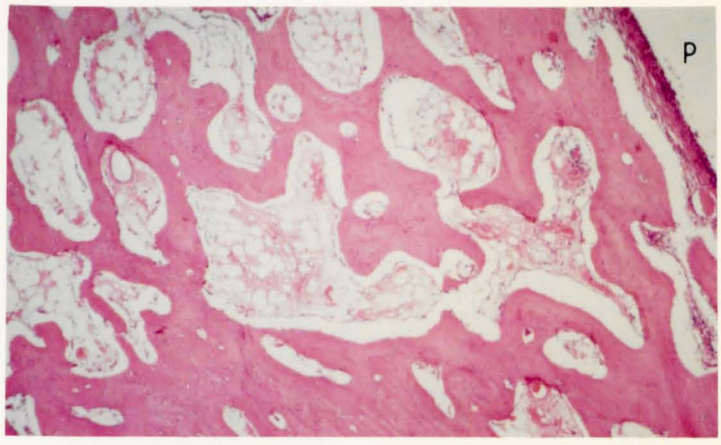
The most striking feature was the marked sub-periosteal new bone formation (see Figure 6.7), which was invariably more extensive than that seen in the infected rod-fixed fractures.

Intramedullary abscesses around the screw tracks were a constant finding (see Figure 6.8). There was always a marked polymorphonuclear leucocyte reaction within the medullary canal beneath the plate. Sequestrum formation was not marked, with only occasional small fragments of dead bone.

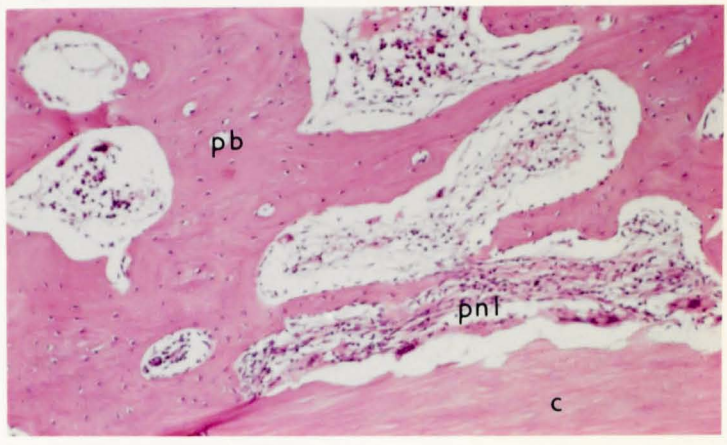
The infective process was always limited to the sections cut from the central block (B - see Chapter 2.5.4) beneath the plate. The histological appearance of both the proximal and distal tibia, away from the plate, was entirely normal (see Figure 6.9).

In three of the remaining thirteen animals, a small area of polymorphonuclear leucocyte infiltration around a screw track was noted (see Figure 6.10). In none of these was there any evidence of sub-periosteal new bone, sequestra or abscess formation. No organisms could be identified on Gram staining of the relevant section. On review of the radiographs of these three rabbits, a small area of lysis around the suspect screw was noted in two cases. There were no other radiological signs of infection. Bacteria had not been isolated at sacrifice and

FIGURE 6.7 : (A) Sub-periosteal new bone formation adjacent to the plate (p). H and E. Magnification x 40. (B) Medium power view (magnification x 100) to show reactive new bone formation (pb) overlying the avascular original cortex (c). There is a polymorphonuclear leucocyte (pnl) reaction around the dead cortex. H and E.



A



B

FIGURE 6.8 : An infected screw track (st). The screw was surrounded by sequestra (s) and debris (d). There is a polymorphonuclear leucocyte (pnl) reaction with a micro-abscess (a). H and E. Magnification x 40.

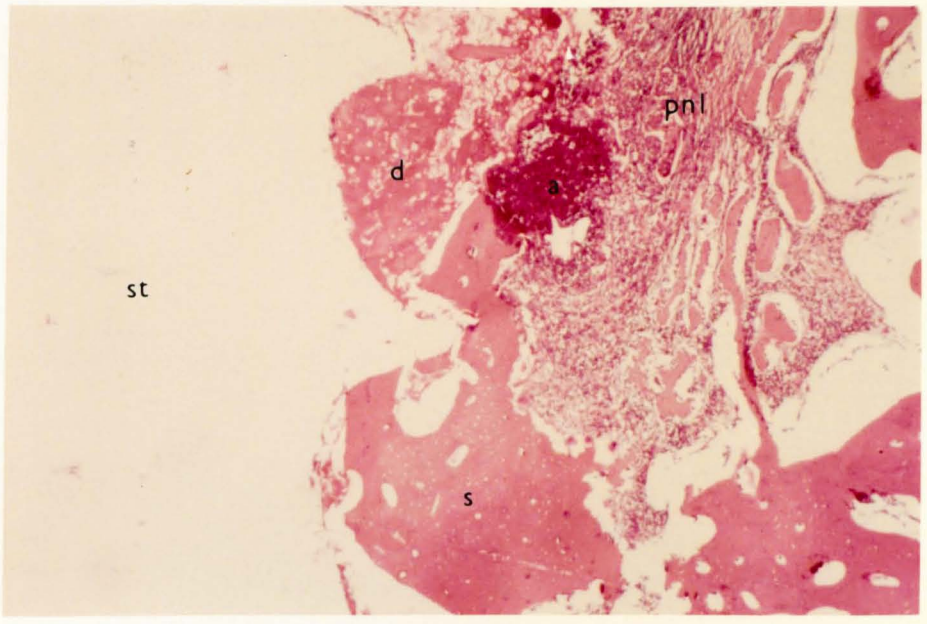

pnl
d
a
st
s

FIGURE 6.9 : Transverse section of the tibia distal to an infected plate. The appearance is entirely normal (compare with Figure 1.2). H and E. Magnification x 100.

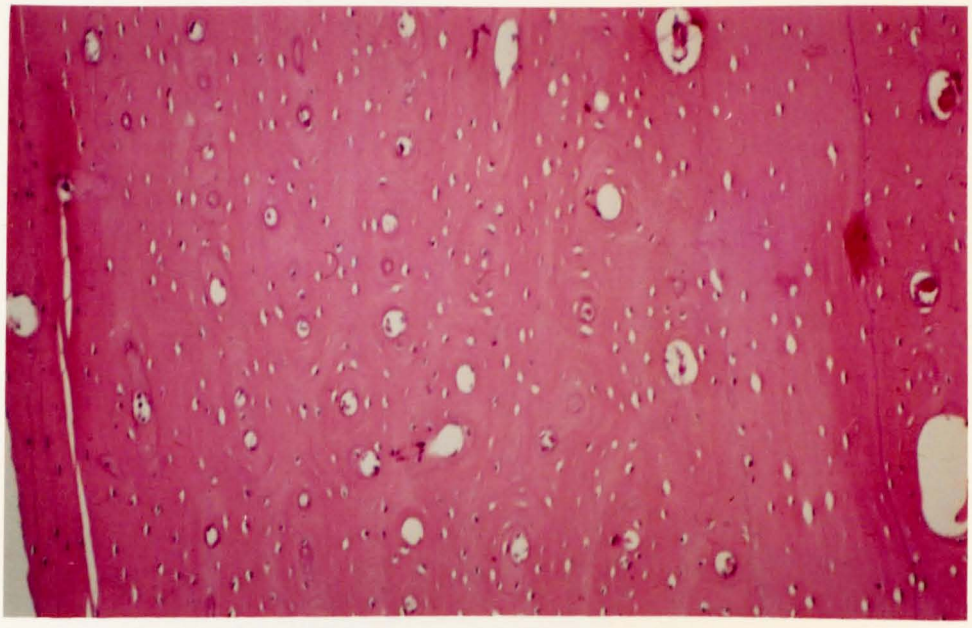
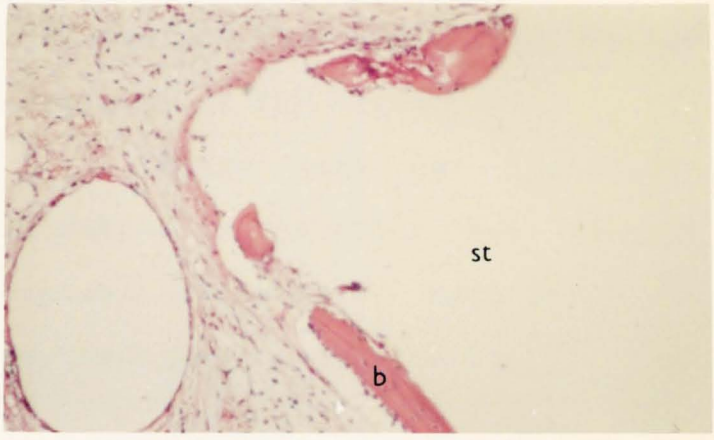
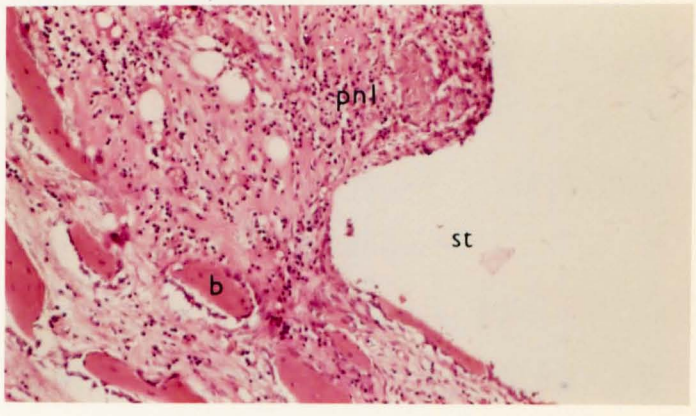


FIGURE 6.10 : (A) The normal appearance around a screw track (st). There is minimal reactive bone formation (b) and no inflammatory response. H and E. Magnification x 100.
(B) Inflammatory reaction around a screw track (st). Although there is polymorphonuclear leucocyte reaction (pnl), this is not as marked as in the infected cases. There are no sequestra, abscesses or debris; there is minimal reactive new bone (b). H and E. Magnification x 100.



A



B

the macroscopic appearance of the tibiae at sacrifice had been entirely normal. These three rabbits were graded "non-infected". The histological appearance was thought to represent loosening of the screw.

There were no histological signs of infection in the other ten animals. All had the typical appearances seen in the control animals, as described in Chapter 3.

Inoculum size :

The infection rate for rabbits inoculated with 10^6 Staphylococci is shown in Table 6.2 and for those animals inoculated with 10^7 Staphylococci in Table 6.3.

In both groups, the infection rate was higher with unstable fixation; but in the animals inoculated with 10^6 organisms this was not significant statistically. However, the difference in infection rates was highly statistically significant in those rabbits inoculated with 10^7 organisms ($p<0.01$).

There was no significant difference in infection rates between the plate-fixed fractures inoculated with 10^6 Staphylococci and those inoculated with 10^7 organisms. In contrast, as the inoculum was increased from 10^6 to 10^7 organisms, the infection rate doubled in the rod-fixed group. This difference in infection

TABLE 6.2 : Osteomyelitis rate with inoculum of 10^6 Staphylococci

GROUP	NO OF RABBITS IN GROUP	NO OF INFECTIONS IN GROUP*
Stable	10	3
Unstable	10	5
	20 rabbits	

* p = NS. Fisher's exact probability test

TABLE 6.3 : Osteomyelitis rate with inoculum of 10^7 Staphylococci

GROUP	NO OF RABBITS IN GROUP	NO OF INFECTIONS IN GROUP*
Stable	10	4
Unstable	11	10
	21 rabbits	

* $0.001 < p < 0.01$ Fisher's exact probability test

rates between the two unstable sub-groups is statistically significant ($0.01<p<0.05$, Fisher's exact probability test).

The increased inoculum had no effect on severity of infection in the unstable group: the clinical, radiological, microbiological and histological pattern was similar in all 15 infected animals. In the plated fractures, there were subjective differences. Radiographic changes were most marked in those rabbits inoculated with 10^7 organisms and abscess formation, with positive culture, was seen at sacrifice in all four of these. Only one of the three infected animals in the 10^6 sub-group had obvious pus with a positive culture. The radiographic changes were also less marked in this group.

6.3 Conclusions :

This study confirms that in an experimental open fracture, the infection rate is 50% less after stable fixation (dynamic compression plate) than after unstable fixation (loose-fitting intramedullary rod). An increasing size of inoculum led to an increased incidence of infection in the unstable group, but had no effect on the infection rate in the stable group.

Despite the high incidence of infection in both rod-fixed and plate-fixed fractures, non-union did not occur. Fracture healing was

always by secondary intention (external callus formation) in the presence of infection.

CHAPTER 7

CEPHRADINE PHARMACOKINETICS IN THE RABBIT

7.1 Methods :

Cephradine is one of the first generation cephalosporins, which are semisynthetic derivatives of an antibacterial compound elaborated by the fungus cephalosporium. It is active against Streptococci and Staphylococcus aureus (including penicillinase producing strains); it is also active against the majority of strains of Escherichia coli, Klebsiella pneumoniae and Proteus mirabilus (Fitzgerald and Thompson 1983). It penetrates well into both normal bone (Parsons et al 1976, Cunha et al 1977) and osteomyelitic bone (Hierholzer et al 1974) in man. Cephradine has been recommended for use as a prophylactic antibiotic in joint replacement surgery (Wicks et al 1981, Brooks and Dent 1984).

Cephradine is excreted mainly through the kidneys and has a low level of oral and parental toxicity in animals (Hassert et al 1973). In normal rabbits, a single intramuscular injection of cephradine (50mg/kg body weight) has been shown to produce high serum levels for up to three hours (Sahn et al 1981). The aim of this part of the study was to confirm that satisfactory serum levels of cephradine could be achieved in male New Zealand white rabbits; and to ensure that there were no long term complication of therapy.

Five rabbits were used; cephradine (Velosef: E R Squibb and Sons) in a dose of 40mg/kg was given by intramuscular injection into the right thigh. Blood was drawn, from a lateral ear vein, every 30 minutes for the first two hours after injection and hourly thereafter until four hours after the injection. The blood samples were immediately centrifuged at 2000 rpm for 25 minutes and the supernatant withdrawn. The serum was deep frozen at -25°C until assayed.

Antibiotic assays were performed using large petri dishes containing Oxoid DST which were seeded with Staphylococcus aureus NCTC 6571. Wells were cut in the agar; standards of 2.5 - 80mg/l of cephradine were made up in rabbit sera and added to the wells. After overnight incubation at 37°C, the zone sizes were read by a plate reader. Assay results were obtained, using a computer program, from the standard curve.

The rabbits were observed clinically for 12 weeks after the cephradine injection, to check for any adverse reactions.

7.2 Results:

The cephradine assays were performed within ten days of the experiment. The results are shown in Table 7.1. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of cephradine for the COM 1

TABLE 7.1 : Serum cephradine levels in mg/l

RABBIT	TIME AFTER ANTIBIOTIC INJECTION (MINUTES)					
	30	60	90	120	180	240
A	105	18	11.5	<2.5	<2.5	<2.5
B	58	12.5	8.0	<2.5	<2.5	<2.5
C	16.5	9.2	5.0	<2.5	<2.5	<2.5
D	38	11	5.0	<2.5	<2.5	<2.5
E	49	14.5	6.0	<2.5	<2.5	<2.5
Mean	53.3	13.0	7.1	-	-	-
SD	±32.8	±3.4	±2.8			
SEM	14.6	1.5	1.3			

Staphylococcus were also measured. Both the MIC and MBC for this strain were 4.0mg/l.

Cephradine is quickly excreted in rabbits and none was detected in any animal at two hours or later. However, despite the rapid fall in serum levels in the first hour, the mean serum cephradine level remained in excess of both the MIC and MBC at one hour after injection. The lowest level measured (rabbit C) was still over twice the MIC and MBC. Even at 90 minutes after injection, the serum cephradine levels were in excess of both the MIC and MBC in all the animals studied.

No side effects of the antibiotic were observed initially. The rabbits continued to feed and gain weight normally. Within the 12 week period of observation no signs of any adverse reaction were seen.

This experiment confirmed that an intramuscular injection of cephradine, in a dose of 40mg/kg body weight, produced adequate serum levels in the rabbit for at least 90 minutes after injection. At one hour after injection, the mean serum cephradine level was over three times the MIC and MBC for the COM 1 organism. The dosage regime was therefore appropriate for the final phase of the study.

CHAPTER 8

PREVENTION OF INFECTION IN EXPERIMENTAL OPEN FRACTURES : THE EFFECT OF ANTIBIOTICS

8.1 Methods :

Fifty-two rabbits were used in this phase of the study. In all, the fractures were stabilised with a loose-fitting intramedullary rod and inoculated with the COM 1 organism in an inoculum of 10^7 bacteria per 0.5 ml buffered phosphate saline. This had been previously shown to give the highest osteomyelitis rate amongst the groups tested (Chapter 6).

The animals were randomly allocated into five groups; each rabbit received one intramuscular injection of cephradine only. The antibiotic was given in a dosage of 40 mg/kg body weight, as this had been confirmed as producing adequate serum levels (Chapter 7).

Animals in each group were given the antibiotic at a different time interval from inoculation : Group A, one hour pre-inoculation; Group B, one hour post-inoculation; Group C, two hours post-inoculation; Group D, three hours post-inoculation; Group E, four hours post-inoculation.

All operations were performed as described in Chapter 3 and Chapter 5 and assessment was carried out using the standard protocols (Chapter 2.5). The rabbits were all sacrified twelve weeks after operation using the technique given in

Chapter 2.4.

Infection was diagnosed if the following criteria were met:

1. Radiological appearance of periosteal reaction, involucrum or sequestrum formation, osteolysis around the implant and sclerosis.
2. Isolation of Staphylococcus aureus (phage type 29) from the culture taken from around the implant at sacrifice.
3. Histological evidence of polymorph-onuclear leucocyte reaction, abscess formation, the presence of gram-positive cocci on Gram staining, bone necrosis and sub-periosteal new bone formation.

8.2 Results :

A total of 52 rabbits were initially used in this experiment. There was one early death : one rabbit in group A failed to make a satisfactory recovery from surgery and was found dead on the second day after operation. No obvious cause of death was found and this rabbit was excluded from further analysis.

During the experiment, a further three rabbits died. One rabbit in group D died at six

weeks after operation; although it had lost weight progressively, no signs of local or systemic infection were found at post-mortem. Another rabbit in group D was found dead eleven weeks after operation; there had been no clinical problems with this animal and no obvious cause of death was found at post-mortem. A death also occurred in a rabbit in group A, at eight weeks after operation; this animal failed to recover from the sedation (Hypnorm - see chapter 2.5.2) used for radiological assessment. This was the only death associated with the use of Hypnorm in the entire series, out of a total of 885 administered doses. These three animals were included in the final analysis as there was complete correlation of the clinical, radiological and histological appearances in all.

The infection rate in each of the five study groups has been compared with that in a group of rabbits who had received no antibiotic. The control group used was the "10^7 rod-fixed group" from the stability phase of the study (see Chapter 6). The results are summarised in Table 8.1.

The "non-infected" rabbits had the typical features of uneventful healing previously described (see Chapter 3). Swelling and erythema settled early and serial radiographs showed healing by external callus formation. There were

TABLE 8.1 : Osteomyelitis in relation to delay in giving antibiotic

GROUP	NO OF RABBITS IN GROUP	NO OF INFECTIONS IN GROUP
No antibiotic	11	10
Antibiotic 1 hour pre-inoculation	10	3*
Antibiotic 1 hour post-inoculation	11	6**
Antibiotic 2 hours post-inoculation	10	5**
Antibiotic 3 hours post-inoculation	10	5**
Antibiotic 4 hours post-inoculation	10	5**
	62 rabbits	

* Difference from no antibiotic group $0.001<p<0.005$) Fisher's exact probability test.

** Difference from no antibiotic group $0.01<p<0.05$) Fisher's exact probability test.

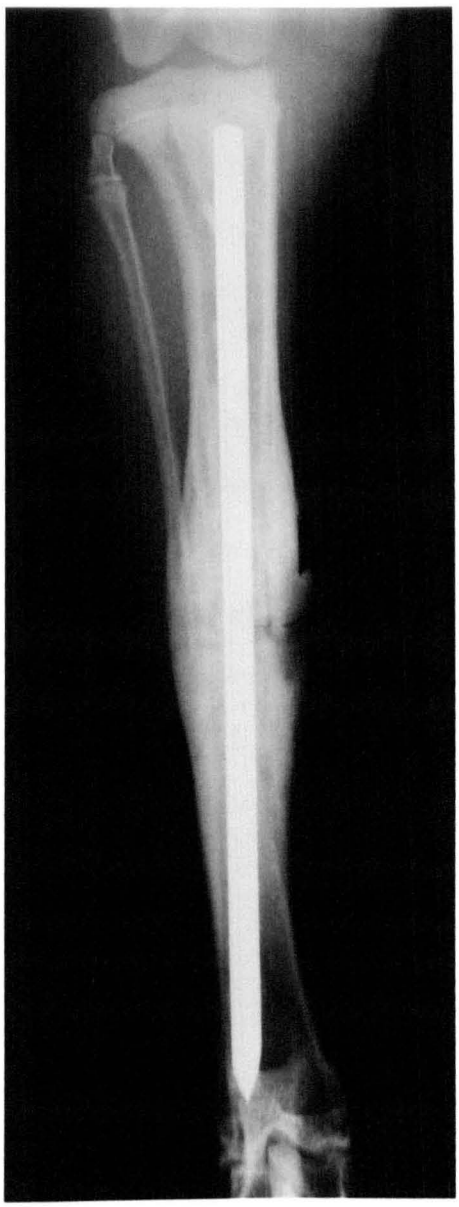
no positive cultures from the swabs taken from around the implants at sacrifice. No histological evidence of infection was seen.

The rabbits graded as "infected" also showed the characteristic signs of osteomyelitis previously observed (see Chapters 5 and 6). There was diffuse generalised swelling of the operated lower leg, with frank abscess formation in three animals. These discharged spontaneously and two healed, but one rabbit was left with a persistent sinus.

No bacteriology swab was taken from the rabbit in group D found dead eleven weeks after operation, but this animal showed clear clinical, radiological and histological signs of infection and was graded as such. Another rabbit in group D that was clinically infected had a negative culture from the swab taken from around the implant at sacrifice. Serial radiographs showed dense sclerosis (see Figure 8.1); and histological examination revealed typical changes of chronic osteomyelitis, with polymorphonuclear leucocyte reaction and intramedullary abscess formation. This was the only definite instance of sclerosing osteitis seen in the entire series.

In one instance, Staphylococcus epidermidis and Staphylococcus aureus were isolated from the culture swab taken at sacrifice. This animal had obvious clinical, radiological and

FIGURE 8.1 : This radiograph was obtained twelve weeks after fracture and inoculation. There is dense sclerosis at the fracture site, with a sequestrum present adjacent to the rod medially. This was the only case of sclerosing osteomyelitis seen.



histological signs of infection. In all the other rabbits graded as "infected" on clinical grounds, only Staphylococcus aureus (of phage type 29) was isolated at sacrifice.

Serial radiographs showed the typical features of periosteal reaction, involucrum formation and late sclerosis and osteolysis in the infected animals. The characteristic histological changes of polymorphonuclear leucocyte infiltration, abscess formation and the presence of involucra/sequestra were seen in all these rabbits.

8.3 Conclusions :

The use of antibiotics will significantly decrease the incidence of infection in experimental open fractures. Although the maximal reduction in osteomyelitis rates was seen when the antibiotic was given before inoculation with bacteria, a 40% decrease in infection rates was still observed when the initial dose of antibiotic was given after bacterial inoculation. This effect was still marked even if the initial dose of antibiotic was delayed up to four hours after inoculation.

CHAPTER 9

DISCUSSION

9.1 Evaluation of the experimental model

My experiments show that it was possible to create a reproducible midshaft fracture of the rabbit tibia, which could be successfully stabilised with either a compression plate or an intramedullary rod. Fractures fixed with a compression plate healed by primary bone union and those fixed with a loose-fitting intramedullary rod healed by external callus formation. Inoculation of the fracture with Staphylococcus aureus caused characteristic changes of osteomyelitis.

Primary bone union in the rabbit was first reported by Rahn et al (1971), who stabilised an experimental osteotomy with a compression plate. The radiological and histological characteristics of bone healing in rabbits after compression osteosynthesis have been further defined by Grieff (1979) and Ashurst et al (1982). These workers obtained serial radiographs and sacrificed animals sequentially between one and 12 weeks after operation. Grieff (1979) reported that when compression plating was used the osteotomy gap varied between ten and 200 micrometres. There was no radiological evidence of callus with such small gaps. Bone healing on histological examination was by gap healing, as reported by Rahn et al (1971). Grieff (1979) saw no primary bone union,

but this may be explained by the fact that he used a less rigid plate than that used by Rahn et al (1971), Ashurst et al (1982) and myself.

Ashurst et al (1982) described the fracture model that I have subsequently used. In their animals, primary bone healing by direct osteoid remodelling was seen. My observations are similar to those of Rahn et al (1971) and Ashurst et al (1982). Moreover, they confirm not only that primary bone union occurs in the rabbit but also that conditions of anatomical reduction and rigid fixation were achieved in nine of the ten animals in my control "plate-fixed group".

Another question relates to the reliability of the criteria used in assessing the diagnosis of osteomyelitis. Clinical signs of abscess formation were uncommon: of the 47 rabbits from all experiments finally graded as "infected", superficial abscesses were only seen in ten animals. Generalised swelling of the the fractured lower leg was invariably seen when osteomyelitis was present, but this is a subjective finding that is difficult to evaluate.

The diagnosis of infection was therefore made on the basis of the microbiological, radiological and histological criteria. These three assessments were qualitative in nature; the aim was to decide whether or not infection was present in a given animal and no attempt was made

to grade either the severity or the extent of infection quantitatively.

The characteristic features of Staphylococcal osteomyelitis in rabbits were described in detail by Crane et al (1977). These workers injected Staphylococci in a concentration of 10^6 organisms together with 0.1ml of 5% sodium morrhuate into the proximal tibial metaphysis. Serial radiographs were then obtained daily for twenty days and eight criteria were assessed: 1) soft tissue swelling, 2) periosteal reaction, 3) osteolysis, 4) sclerosis, 5) knee joint effusion, 6) obliteration of the medullary cavity, 7) sequestrum formation and 8) involucrum formation. These eight criteria were graded on a four point scale and summated to produce a daily pathologic score. The animals were sacrificed at varying intervals after inoculation. Serial radiographs correlated well with the progressing pathological findings.

The radiological pattern of infection in my series is similar to that reported by Crane et al (1977), but changes appeared later than in their animals. Crane and his co-workers reported periosteal reaction and lysis formation as early as the fifth day after inoculation with bacteria, with marked abscess and sequestrum formation by day 20. In contrast, periosteal reaction and involucrum formation were not seen until four to

six weeks after operation in my rabbits and lysis around the implant was a late feature. The severe early radiological signs described by Crane et al (1977) were probably related to their use of sodium morrhuate as a sclerosing agent. This produced aseptic necrosis of bone, which is an ideal site for bacterial proliferation protected from the immune defence systems.

The radiological pattern of infection in my series is also very similar to that described by Rittmann and Perren (1974); they studied infection of experimental osteotomies in sheep, fixed with compression plates. They reported the appearance of periosteal reaction between three and five weeks, followed by the formation of sequestra between five and seven weeks. Sclerosis and osteolysis were late features.

Rittmann and Perren (1974) also graded the severity of the radiological findings on a three point scale, but did not attempt to quantify further. The findings of Rittmann and Perren (1974) and Crane et al (1977) were used in selecting the radiological criteria in my work. Crane et al (1977) reported no radiographic changes in the control rabbits, in which there were no histological evidence of infection. The aim of the radiological assessment in my animals was only to diagnose when infection had developed. Therefore, the radiological criteria were only

graded as "present" or "absent", with the exceptions of callus formation and periosteal reaction; these were graded on a four point scale, derived from their extent along the tibial shaft.

The typical radiological changes of osteomyelitis in my rabbits were: periosteal reaction, involucrum formation, sclerosis and osteolysis around the implants. These radiographic changes were only seen in those animals in which there were histological signs of osteomyelitis; they were not seen in rabbits without microbiological or histological signs of infection. There was complete agreement between the two observers in the subjective radiological assessment of both the pattern of fracture healing and the diagnosis of infection in all the animals.

The histological features of bone infection in rabbits were described in detail by Crane et al (1977), who also confirmed that isolation of bacteria from bone was invariable when there was histological evidence of inflammatory exudates and micro-abscesses.

In my entire series, 47 rabbits were finally graded as "infected"; and there was a triad of positive culture, typical radiological changes and characteristic histology in 43 of these animals. The other four rabbits (each of which has been described previously) each had negative bacteriological culture, but typical radiological

and histological appearances of infection. The identification of gram-positive cocci in these four rabbits was the final confirmation of osteomyelitis. My results confirm the work of Crane et al (1977) on the correlation of bacteriological, radiological and histological assessments in the diagnosis of Staphylococcal osteomyelitis in the rabbit.

There were 96 rabbits graded as "non-infected" from my experiments. In all except three, there was a negative bacteriological culture, with no radiological or histological features of osteomyelitis. These three rabbits have been described previously (Chapter 6.2): a polymorphonuclear leucocyte reaction was seen around a screw track at histological assessment. However, although in two rabbits there was minimal radiological evidence of lysis around the suspect screw, there were no other radiological or histological features of infection and no organisms were cultured. The equivalent sections were examined by Gram's method and no bacteria were seen. These three rabbits were therefore included in the "non-infected" group.

My results show that the radiological, microbiological and histological assessment described can be confidently used to diagnose osteomyelitis in fractures of the rabbit tibia, after stabilisation with metallic implants.

An open fracture consists of : 1) a fracture, 2) the wound and 3) the bacteria contaminating the wound. Although the bacteria were not inoculated until after wound closure, my experimental model is analagous to an open fracture in man. The method has a standard surgical approach, which resulted in a similar wound and local trauma in each animal and the fracture created was totally reproducible. Ideally, bacterial inoculation should occur at the time of fracture. However, this could have lead to loss of part of the bacterial inoculum during the latter stages of the procedure. The needle placed with its tip at the fracture site allowed reliable and direct inoculation of the fracture with a measured number of bacteria.

Previous work has suggested that osteomyelitis could be induced in rabbits by inoculating a rod-fixed fracture with only 10^5 Staphylococci (Friedrich and Klaue 1977). I was unable to confirm this observation and found that a minimum of 10^6 organisms was necessary before infection occurred. The osteomyelitis rate increased with the use of a larger inoculum of 10^7 organisms. Although the size of such inocula may seem large, it has been found that in human skin wounds, an inoculum of over 10^5 organisms per gram of tissue is required before infection is likely (Marshall et al 1976, Cooney et al 1982).

There was a low mortality in my experiments: only nine animals (6.3%), of the 143 in the operative studies, died or were sacrificed before completion of the experiment. Many workers do not quote their failure rate in their animal experiments, but my failure rate of 6.3% is one of the lowest reported when compared with previous work on osteomyelitis and fracture healing in rabbits (see Table 9.1). There were no intra-operative anaesthetic deaths; and there was only one death related to the use of Hypnorm as sedation during radiography.

This low mortality in my animals is mainly due to the skill and expertise in anaesthesia of the staff of the Animal Unit at the Queen's Medical Centre, Nottingham. Although induction of anaesthesia with intravenous barbiturates has been extensively used in rabbits, this has been associated with problems of respiratory arrest and death (Ashurst 1984). My work confirms the safety of gradual gas induction by mask in the rabbit; and that intramuscular Hypnorm is a safe agent for sedation.

9.2 The effect of fracture stability

My infection rate after compression plating was 40% less than that in fractures fixed with intramedullary rods. Two questions are posed: firstly, is this difference solely due to

Table 9.1 : Failure rate (defined as death or sacrifice before completion of the experiment) in studies of fracture healing or osteomyelitis in rabbits.

Study	Type of study	No of rabbits entered	No of failures	% failure rate
Crane et al (1977)	Osteomyelitis	45	7	15.6%
Southwood et al (1985)	Osteomyelitis	120	27	22.5%
Norden (1970)	Osteomyelitis	64	19	29.7%
Terjesen (1984)	Fracture healing	58	8	13.8%
Grieff (1979)	Fracture healing	35	10	28.7%
Rahn et al (1971)	Fracture healing	8	0	0.0
Ashurst et al (1982)	Fracture healing	45	6	13.3%
Dwyer (1970)	Fracture healing	36	12	33.3%
Worlock (present study)	Fracture healing & Osteomyelitis	143	9	6.3%

the difference in stability between the two groups; and secondly, why does fracture stability have an effect on infection?

With regard to the first of these questions, the experimental model was so designed that the only variable was the method of fixation. Dynamic compression plating represented the more stable form of metallic fixation; and a loose intra-medullary rod, the less stable form. Both implants were made of the same stainless steel (type AlSl 316L).

The mechanical study in Chapter 3 showed the difference in stability between these two methods of fixation. This was also confirmed by the observed pattern of fracture union : the stable fractures healed by primary bone union and the less stable by external callus formation.

The trauma to soft tissues and to bone, caused by the creation of the experimental fracture, was similar in both groups. However, each method of fixation is believed to have a different effect on the blood supply to the tibia. Could damage to the vasculature and bone necrosis be responsible for the observed difference in infection rates?

Although it has been reported that intramedullary nailing will cause avascular necrosis of the inner two-thirds of the cortex (Trueta and Cavadias 1955), other workers have not

confirmed this finding. Gothman (1960b,1960c) found no histological evidence of avascular necrosis after intramedullary nailing of the rabbit tibia; he reported that the vascular response of the periosteum was capable of maintaining cortical vascularity after damage to the medullary arteries.

Rhinelander (1974) has also shown that a loose-fitting intramedullary rod will only cause cortical necrosis where it is in contact with the endosteal surface; and that there is an excellent vascular supply to cortical bone surrounding a loose-fitting rod (Rhinelander et al 1967). Cortical necrosis will also occur underneath a plate (Olerud and Danckwardt-Lilliestrom 1968, Rhinelander 1974); and it may involve the full thickness of the bone.

These observations have been confirmed in my experiments. Cortical bone necrosis was seen both beneath plates and where intramedullary rods were in contact with the endosteum. However, there was no evidence of avascular necrosis in the remaining cortex not in contact with an implant. Cortical necrosis was not as extensive in depth as previously reported (Trueta and Cavadias 1955, Rhinelander 1974). My histological observations are in accord with dynamic studies, which suggest that both compression plating and intramedullary nailing affect the blood flow at the fracture site

equally (Rand et al 1981).

Intramedullary nailing and compression plating will cause damage respectively to the medullary and the periosteal vascular systems; the undamaged vascular system may then increase the blood supply to the entire cortex. Neither the previous work nor my own histological observations support the contention that increased vascular damage (and hence cortical necrosis) after intramedullary nailing might be responsible for the increased infection rate in this group.

The other variable between the two groups is a difference in stability. This leads to a second question: why does increased stability at the fracture site reduce the infection rate?

Infection in bone depends on an interaction between bacteria and host. Considerable attention has been directed to the nature and control of bacteria, with little attention paid to the tissues inoculated (Brown 1973). Selye (1956) reports that Pasteur said on his deathbed: "The germ is nothing, it is the terrain in which it grows which is everything".

Khan and Pritzker (1973) stated that three conditions are necessary for the development of osteomyelitis. These are:

1) The presence of bacteria.

2) Factors favouring localisation of the bacteria : these include firstly, vascular stasis

which may be important in acute haematogenous osteomyelitis; and secondly, an open fracture which localises the bacteria to the fracture ends.
3) Factors favouring bacterial proliferation : these include blood clot, exudate formation and tissue necrosis.

Stability is clearly beneficial to revascularisation; both Rhinelander et al (1967) and Ganz et al (1970) have confirmed that blood vessels can be seen crossing an experimental osteotomy with a few days of operation, provided that rigid internal fixation has been maintained. However, this revascularisation of the fracture site occurs days after injury; and it seems likely that the first few hours after inoculation are critical in determining the outcome (Miles et al 1957, Polk and Miles 1973).

It has been suggested that stable fixation of a fracture leads to improved tissue microperfusion in the adjacent area (Chapman 1982). This would bring cellular and humoral defences into tissues contaminated with bacteria. This is an attractive theory, but, as yet, there is little evidence to support it. Anatomical reduction and stabilisation of a fracture will reduce tissue dead space and decrease the chance of haematoma formation. Conversely, instability at the fracture site will cause local necrosis of surrounding soft tissues and promote exudate

formation. All these factors will allow bacterial proliferation (Kahn and Pritzker 1973, Chapman 1982).

In experimental dermal and muscle infections, there is extensive destruction of the inoculum by the local defences during the first four hours after inoculation (Miles et al 1957, Polk and Miles 1973). Such local defences can be inhibited, and the subsequent infection "enhanced", by local ischaemia and local tissue necrosis. If this hypothesis of the "decisive period" is accepted, the effect of stable fixation must occur during this period (at least in part). The exact mechanism remains to be clearly defined, but an improvement in microperfusion and the prevention of both exudate formation and tissue necrosis would allow the local defence mechanisms to act optimally (Mader and Cierny 1984).

Indirect evidence for the theory that stabilisation of the fracture improves tissue microperfusion is given by my observations on the size of bacterial inoculum. Thus, increasing the inoculum from 10^6 to 10^7 organisms had no effect on the infection rate after plate fixation of fractures. However, the infection rate doubled in fractures stabilised with intramedullary rods when the inoculum was increased by the same amount. Stable fixation may allow local defence mechanisms to deal adequately with larger numbers of

bacteria; but these defences may be overwhelmed when there is continuing local ischaemia and necrosis, as in conditions of unstable fixation.

9.3 The effect of antibiotic therapy

My work shows that in experimental infections of bone, the maximal reduction in infection rate was seen if the antibiotic was given before inoculation with bacteria. However, a significant reduction in the infection rate still occurred if the initial dose of antibiotic was delayed until after inoculation. In my experience there was no progressive diminution of effect with increasing delay in giving the initial dose of antibiotic. This antibiotic effect was as marked after four hours delay as after an hour delay.

My findings are in contrast to previous work on experimental infection in skin wounds. Both Miles et al (1957) and Burke (1961) reported that the effect of antibiotics in preventing infection in experimental dermal incisions was maximal if adequate levels of antibiotic were present in the tissues before contamination with bacteria. Burke (1961) has also shown that the effect of antibiotics became progressively less as the initial dose was delayed after inoculation with bacteria. If the initial dose was delayed three or more hours after inoculation, antibiotics

had no effect on the outcome.

The warning of Bowers et al (1973) now seems very appropriate : "Caution should be exercised in extrapolating data on prophylaxis applied to soft tissue wounds to that for wounds in bone". Bone is different from soft tissues : infection is difficult to establish without the presence of other factors (Kahn and Pritzker 1973) and it is difficult to eradicate once established.

The first local response to wounding and bacterial contamination is an inflammatory reaction. There is exudate formation and leucocyte migration into the wound (Mader and Cierny 1984). There is an extensive "kill" of bacteria during the first few hours after inoculation by these local defence mechanisms, which cease to operate after this time (Polk and Miles 1973). The subsequent course of the infection is determined by the number of organisms which survive this period. In man, the local defences can deal with an inoculum of up to 10^5 organisms per gram of tissue (Marshall et al 1976, Cooney et al 1982); with larger numbers of bacteria, the local defences are overwhelmed and infection results.

Antibiotics will play a part in augmenting local defences by participating in this early "kill" of bacteria. Two factors may limit the effectiveness of antibiotics. Firstly, if there

is massive contamination of the wound with very large numbers of organisms, there may not be enough antibiotic available to destroy a significant number of bacteria. Secondly, the antibiotic may be prevented from contact with the bacteria. Antibiotics will not penetrate necrotic tissue, the presence of which will hinder their action. It has also been shown, in experimental open wounds, that a fibrinous coagulum forms which surrounds the bacteria and protects them from antibiotics (Edlich et al 1973).

Edlich and his co-workers have also demonstrated that the effective period of preventative action of antibiotics can be extended by up to 24 hours, when the wound is primarily closed. Primary wound closure is not associated with the production of a fibrinous coagulum; and this may account, in part, for the prolonged duration of action of antibiotics that I have demonstrated in experimental bone infections.

However, the nature of the interaction in bone between bacteria, local defence systems and antibiotics requires further investigation. Bowers et al (1973) showed in an experimental infection of the canine femur, that antibiotics would prevent infection if given before bacterial inoculation; but they had no effect if the initial dose was delayed six hours after inoculation. All wounds were primarily closed in their series and

they also demonstrated penetration of antibiotic into haematoma in high concentrations. The work of Bowers et al (1973) and my own observations suggest that the prolongation of the "golden period" of antibiotic action in experimental bone infections is unrelated to primary closure of the wound alone.

Until further evidence is available, the most likely explanation is that antibiotics reduce infection in experimental bone infections by reducing the number of viable bacteria below a critical level. Local defence systems are then able to deal with the remaining organisms.

9.4 Clinical implications :

My experiments on fracture stability support the concept that the primary stabilisation of open fractures is beneficial in man. Which method of stabilisation should be used is less clear. Doubt has been expressed by Hicks (1957) on the relationship between metal and bone in the development of infection. However, recent studies have demonstrated that the presence of a variety of materials (including stainless steel and polymethyl-methacrylate) in experimental wounds will reduce the number of bacteria necessary to cause infection (Zimmerli et al 1982, Christensen et al 1983, Southwood et al 1985).

It would seem appropriate to restrict the

use of compression plating to Type I and Type II open fractures. The soft tissue damage and degree of bacterial contamination is less than in Type III fractures; and infection rates in Type I and Type II open fractures are consistently low (Gustilo and Anderson 1976, Gustilo et al 1984). External fixation with a rigid system may be a better alternative in those injuries with extensive damage to soft tissues and bone (Clifford et al 1986). However, whether compression plating or external fixation is used, the fracture should be stabilised as rigidly as possible in the early stages to allow unhindered soft tissue healing.

Appropriate antibiotics should be routinely used in the management of open fractures. The use of antibiotics is supplementary to the accepted surgical management of both early and thorough cleansing of the wound and excision of necrotic soft tissue. Although antibiotics should be given as early as possible afer injury, my experimental work suggests that benefit can still be gained if the initial dose is delayed for up to four hours after injury.

The choice of antibiotic is dictated by the potential bacterial contamination. A first generation cephalosporin is suitable for Type I and Type II fractures. The pattern of bacterial pathogens in Type III open fractures has altered

over the past 30 years (Gustilo et al 1984). Gram-negative bacteria are more commonly seen; and a combination of a cephalosporin and an aminoglycoside, or a third generation cephalosporin alone, is currently recommended for Type III fractures. If anaerobic contamination is suspected in any open fracture, then metronidazole should be added to the antibiotic regime.

The potential effect of antibiotic therapy is diminished in experimental wounds when these are left open (Edlich et al 1973). However, clinical experience suggests that the correct management of wounds associated with open fractures is delayed closure; this practice may account for some loss of efficacy when antibiotics are used in man. Rodeheaver et al (1978) have shown in experimental dermal wounds, that treatment of the wound surface with proteolytic enzymes disrupts the fibrin coagulum; this exposes the bacteria to the action of the antibiotic. The topical use of enzymes is associated with a significant increase in the concentration of antibiotics in the wound and a decrease in the rate of infection. This concept needs further investigation in man.

9.5 Future research :

The experimental model of post-traumatic osteomyelitis which I have developed may be useful

in investigating the prevention and the treatment of infection after fractures.

There are two studies related directly to my work, which need early evaluation. Firstly, the best method of stabilisation should be determined for this experimental model. I hope to develop a suitable external fixator for the rabbit tibia, of a similar rigidity to the dynamic compression plate. This would allow the foreign body effect of the plate to be eliminated from the experiment. Secondly, the duration of action of antibiotics needs to be further defined. It is not clear what is the maximum delay, in giving the initial dose of antibiotic, that will still result in a reduction in infection rate.

The model has now been modified to allow stabilisation of an osteotomy with a three millimetre gap using a dynamic compression plate. This is being used to study the fate of an autologous cancellous bone graft in the presence of infection. The effect of the bone graft on the outcome after inoculation with bacteria is also being studied; the clinical results of cancellous bone grafting of infected non-unions via the open wound (Roy-Camille et al 1976, Papineau et al 1979) sugest that the graft itself has a beneficial effect.

I have been able to demonstrate that stabilisation of the fracture and delayed

antibiotic therapy will reduce the incidence of experimental bone infection. The reasons for these effects are not clear. Later, it will be necessary to define how the local defence mechanisms in bone have been modified by these two factors.

The findings from animal experiments cannot be directly applied to man, although some biological principles are common to man and animals. There is a need to assess different methods of treatment of human fractures by controlled clinical trials. Neither the effect of stability nor the effect of antibiotics have yet been properly studied in open fractures in man. This deficit in our knowledge must be corrected if the infection rate after open fractures is to be further reduced.

9.6 Conclusion

There are three goals in the management of open fractures : preventing infection; fracture healing; and full functional recovery of the limb. Infection is usually considered to be the cause of both non-union and poor function after such injuries. The aim of treatment of an open fracture is to create the ideal biological environment for the prevention of infection.

The experimental model I have developed provides controlled conditions for the study of

post-traumatic osteomyelitis. My work suggests that fracture stability and antibiotic therapy are two important factors in preventing infection. The findings support the concept of stabilising open fractures in man. Moreover, they suggest that appropriate antibiotics, administered systemically, should be routinely used in the management of such open fractures.

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