International Orthopaedics (SICOT) (1997) 21: 267–273

International
Orthopaedics

The reaction to nailing or cementing of the femur in rats

A microangiographic and fluorescence study

U. E. Pazzaglia[1], **L. Andrini**[2], **A. Di Nucci**[3]

[1] Facoltà di Medicina e Chirurgia, Università di Brescia, Brescia, Italy
[2] 2a Facoltà di Medicina e Chirurgia, Università di Pavia, Pavia, Italy
[3] Istituto di Farmacologia 2, Università di Pavia, Pavia, Italy

Accepted: 29 January 1997

Summary. *Bone reaction to cement and to a cementless stem was studied in the rat femur with histological fluorescence and microangiographic techniques. Periosteal and endosteal apposition, and consequent remodelling, appeared as a reaction to reaming rather than caused by cement or a cementless stem. Every change in bone began with proliferation, progression and orientation of the vessels. Endosteal apposition was absent in cemented femurs because the entire medulla was occupied by the acrylic cement, but remodelling of the subendosteal cortex followed medullary revascularisation which was far advanced after 90 days. In cementless stems, endosteal apposition of primary woven bone and remodelling was the basis for bony ingrowth and anchorage through bony bridges. Our results suggest that the pattern of blood supply is relevant to the structural organisation of mature lamellar bone around the implant. Cemented stems have maximum anchorage and stability as soon as they are inserted, but this decreases with time as revascularisation occurs. Cementless stems can reach maximum integration later after insertion, and revascularisation is less critical because they usually do not fill the canal completely.*

Résumé. *A l'aide des techniques histologiques microangiographiques et à fluorescence, la réaction osseuse lors de l'introduction endomédullaire dans le fémur d'un rat de ciment acrylique et de tiges non cimentèes a été étudiée. La réaction osseuse du périoste interne et externe et la reproduction osseuse ou le remodelage osseux paraissent être des phénomènes dûs au procedé de fraisage du canal médullaire plutot qu'à la réaction spécifique de la cimentation ou à l'introduction d'une tigue non cimentée. Tout changement de l'os, aussi bien en apposition qu'en remodelage implique une prolifération, une progression et une nouvelle orientation des vaisseaux. L'apposition du périoste interne n'apparait pas pour les fémurs cimentés et on observe un remodelage de l'endoste dû au phénomène de revascularisation médullaire; ce phénomène est trés evident aprés 90 jours. Pour les tiges non cimentées, par contre, l'apposition du perioste interne de la matrice osseuse primaire et le successif remodelage permettent la repousse osseuse sur la surface de l'implant, en le soudant à l'aide de ponts osseux à la superficie du périoste interne. Nous pouvons donc dire que d'aprés nos études, les tiges cimentées garantissent un ancrage optimal dés son application mais il est évident qu'au cours du temps, à cause du phénomène de revascularisation l'ancrage diminue. Les tiges non cimentées atteignent le maximum d'ancrage longtemps aprés l'implantation et le procedé de revascularisation est beaucoup moins critique vu que l'implant, habituellement ne remplit pas complétement le canal médullaire.*

Reprint requests to: U. E. Pazzaglia, Clinica Ortopedica Università di Brescia Spedali Civili, I-25100 Brescia, Italy

Introduction

The main arterial supply to the diaphysis of long bones is provided by the marrow arteries which pass outwards from the endosteal surface into the

cortex, whereas the periosteal vessels only supply its outer part [2, 3, 8, 13, 20, 23]. Abundant anastomoses between the endosteal and periosteal vessels have been described [25].

Reaming the medullary canal damages the marrow vessels and changes the direction of their blood flow, reversing it from centrifugal to centripetal [17], producing varying degrees of endosteal bone necrosis [11, 12, 26]. This is relevant to the repair of fractures, and also to the fixation of prosthetic stems since the distal part has to fit the canal and reaming is required to insert both cemented and cementless implants. If the canal is filled with cement or a stem, both venous drainage from the diaphysis [14] and medullary arterial revascularisation are obstructed with changes to the pattern of the blood supply [4, 7, 11, 21, 24].

Anatomical and mechanical factors affect the type of bonding and the structure of the interface. In this study, we have reproduced the conditions of cemented and cementless implants in the rat's femur and compared them.

Materials and methods

Twenty-four Wistar white rats (Stefano Morini, S Polo d'Enza, Reggio Emilia, Italy) weighing about 400 g were used. Under barbiturate anaesthesia, both knees were opened through a lateral parapatellar incision and a 3 mm drill hole was made in the intercondylar notch. The canal was then reamed by hand with a specially designed reamer from 1.5 to 3 mm diameter depending on the individual's size. The canal was then repeatedly irrigated with sterile saline under pressure to remove debris and fat. In group A, the right femur was filled with Sulfix cement (Howmedica, Rutherford, USA) through a 3 mm catheter and a specially designed syringe, a miniature version of a cement gun. In group B, a stainless rod was implanted in the left femur, 2 cm long and the same diameter as the reamer. The rod was introduced with gentle pressure as far as it would go.

In the control rats, the right femur was reamed, but no rod or cement inserted (Group C); the left femur was not touched (Group D).

The rats were injected daily with 30 mg/kg of tetracycline intraperitoneally for the first 30 days of the study and the long term group received the same dose daily for the last 15 days.

Four implanted rats and 2 controls were killed at intervals of 7, 15, 30 and 90 days with an overdose of barbiturate. The vascular tree of the hind limbs was washed through with heparinised saline by a cannula in the abdominal aorta. The fluid was collected from the inferior vena cava which was then ligated to increase the perfusion pressure of the solution (50% Indian Ink) injected into the aorta. Both femurs were dissected from the soft tissues and fixed in 10% formalin.

Radiographs were taken and then 3 mm thick sections were cut, starting from the intercondylar region, with a low speed saw (Isomet, Buheler Ltd, Lake Bluff, III, USA). The plane of the cut was perpendicular to the longitudinal axis of the bone and the sections were embedded in Technovit resin (Kultzer & Co GmgH, Werheim, Germany). Thin sections were prepared with a cutting-grinding device (Exact Apparatebau, Norderstadt, Germany).

Unstained sections, 50 μ thick, were examined in a bright field and in incident fluorescent light with a Leitz Aristophot microscope. Selected sections were thinned to 10 μ, stained with haematoxylin-eosin (HE) and examined in a bright field.

Continuous labelling with tetracycline for the first 30 days allowed a semi-quantitative evaluation of fluorescence at 7, 15 and 30 days (Table 1) with regard to the following features:

(a) Metaphyseal appositional activity, and endosteal and periosteal apposition, in groups A, B and C were graded 0 when there was no increase in the width of the fluorescent bands compared to the controls (group D) for 7 days, or to the previous time interval in the same group. The width of the bands was assessed with a reticule in the eyepiece of the microscope. The value for each section was the mean of 4 measurements performed on 2 orthogonal axes of the femoral section (anterior/posterior and lateral/lateral). Apposition was graded + in the opposite case. Only differences above 10% were considered significant.

(b) Periosteal remodelling was graded according to the percentage of dark zones inside the fluorescent band: 0 = less than 10%; * = 10 to 30%; ** = more than 30%. The images were digitalised on a graphics tablet (Summasketch Plus, Summagraphic, Fairfield, CT, USA) coupled with an IBM AT personal computer. We developed a programme to calculate the percentage of dark areas.

(c) Cortical remodelling was graded 0 when fewer than 5 osteones were labelled, and @ when more than 5 were labelled.

No quantification was possible at 90 days because of the advanced stage of remodelling.

Table 1. Semiquantitative evaluation of fluorescence in the groups at intervals of 7, 15 and 30 days after continuous labelling with Tetracycline (30 mg/kg/day) intraperitoneally

Groups	Metaphyseal appositional activity			Endosteal apposition			Periosteal apposition			Periosteal remodelling			Cortical remodelling		
Days	7	15	30	7	15	30	7	15	30	7	15	30	7	15	30
A) Cemented	+	+	+	0	0	0	+	+	0	0	**	*	0	0	@
B) Cementless	+	+	+	+	+	+	+	+	0	0	**	*	0	0	@
C) Reaming alone	+	+	+	+	+	+	+	+	0	0	**	*	0	0	0
D) Normal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 Absent; + * @ Present; ** Present intense

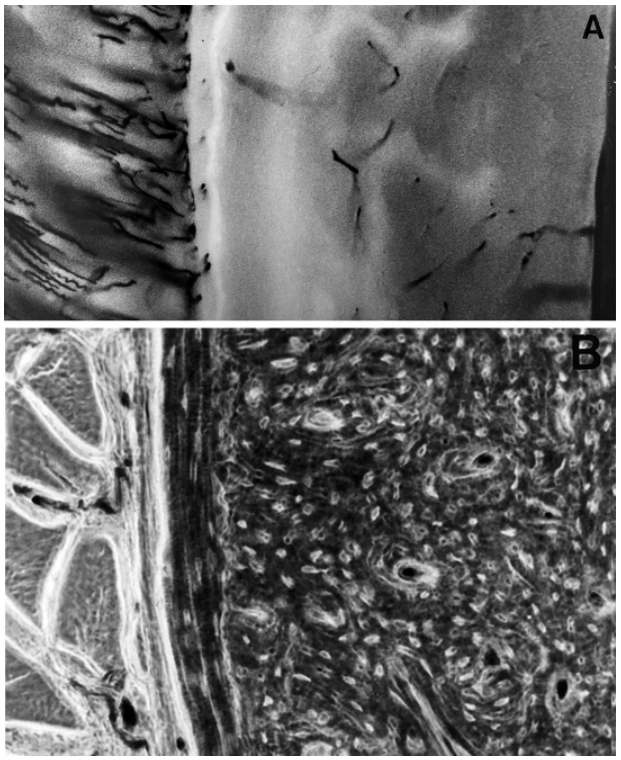

Fig. 1. Unreamed control femur (group D). **a** The normal pattern of periosteal vessels; the thin fluorescent band represents normal periosteal apposition and there is no activity on the endosteum (×100, fluorescence). **b** The periosteal apposition is lamellar and concentric to the diaphyseal circumference; remodelling is the next step (×100, phase contrast)

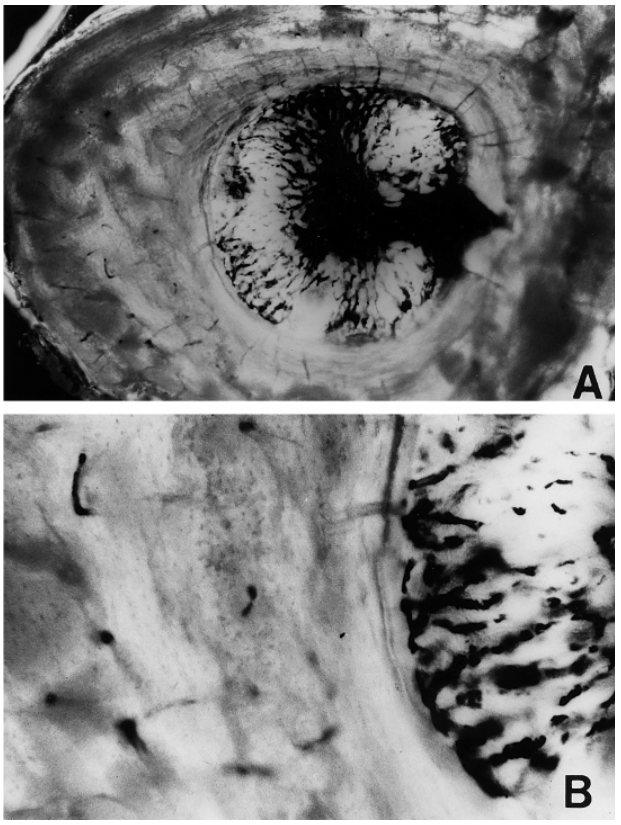

Fig. 2 A, B. Unreamed control femur (group D). **A** Endosteal supply from the medullary artery (×12, unstained, vascular tree injected). **B** Pattern of the distribution of vessels to the endosteal surface; injected vessels are present in the Volkmann canals of the cortex (×100, vascular tree injected)

Results

Control unreamed femurs (group D)

These did not show a band of fluorescence on the endosteal surface of the canal. A complete thin band was present on the periosteal contour of the diaphysis corresponding to normal bone growth in rats of this age. Vessels reached the outer surface of the cortex and had a regular radial pattern from muscles to periosteum. The larger vessels were straight or slightly wavy and ended in the periosteum. Smaller straight branches penetrated the bone through the Volkmann and Haversian canals (Fig. 1 a). A row of osteoblasts was laid down on circumferential and concentric lamellae on the outer cortex (Fig. 1 b).

A similar radial pattern from branches of the medullary arteries was present on the endosteal surface (Fig. 2 a, b). Labelled osteones were rarely seen in the thickness of the cortex.

In the distal femur, a larger number of epiphyseal and metaphyseal trabeculae had labelled borders because of the greater turn-over of cancellous bone.

Periosteal reaction of implanted femurs (groups A and B) and control reamed femurs (group C)

At 7, 15 and 30 days, similar periosteal reaction was found in groups A, B and C, so they are considered together:

(a) *At 7 days*

A thick layer of periosteal bone was present quantitatively and qualitatively compared to group D femurs, the width of the former being 15 times the latter. Groups A and B showed the same radial pattern of periosteal vessels, but they were enlarged, congested and twisted. A large amount of primary woven bone was laid down between the vessels; labelling was not distributed uniformly and there was a shaded irregular contour

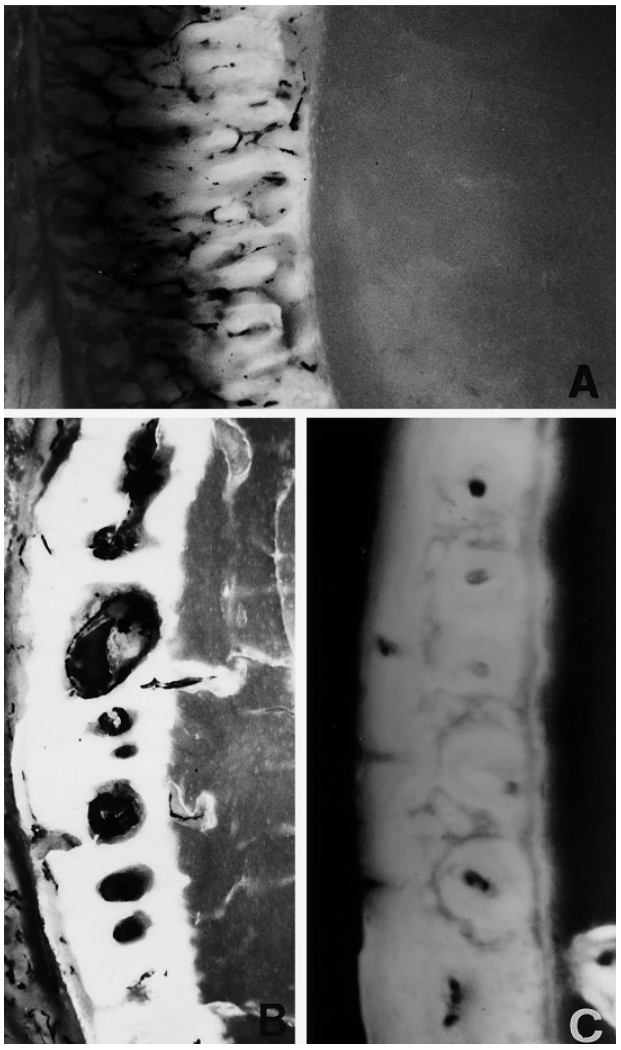

Fig. 3 A–C Reamed cemented femur (group A). **A** At 7 days, the periosteal vessels are hypertrophied and congested and the outer layer of periosteal apposition is 15 times thicker than in unreamed controls (D). The outer part of the periosteal woven is not labelled by tetracycline because the bone is not yet calcified due to the rapid apposition (×100, fluorescence). **B** At 15 days, the rapid periosteal apposition has ceased; all the new bone is labelled and vessels have penetrated the woven bone forming lacunae (×80, fluorescence). **C** At 30 days, there is remodelling of periosteal apposition with injected vessels in the centre of new osteones (×80, fluorescence)

because the outer layer was not labelled at all (Fig. 3a). In contrast, periosteal apposition in group D showed a more homogeneous compact and demarcated band of fluorescence.

(b) *At 15 days*

The rapid apposition of woven bone in groups A and B had diminished; the outer layer of periosteal apposition is smooth and well demarcated, the

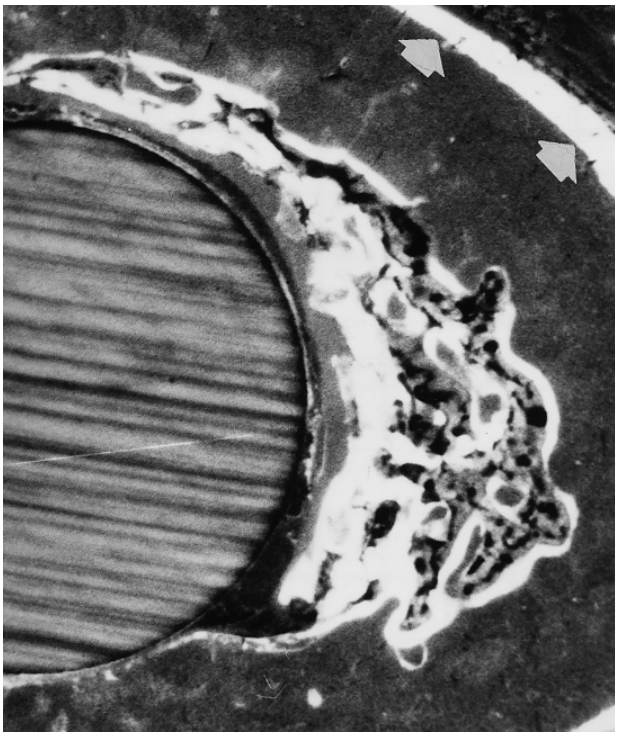

Fig. 4. Reamed cementless implant (group B). At 30 days, labelled woven bone fills the medullary cavity between implant and endosteal surface; it is undergoing remodelling and a network of injected material is present inside the labelled bone. *Arrows* show a band of periosteal apposition (×40, fluorescence)

periosteal vessels are normal, and fluorescence is more evenly distributed. The width of periosteal apposition is greater than in group C, but the main difference is the presence of resorption lacunae appearing as spherical unlabelled holes with an injected vessel in the centre (Fig. 3b). Turnover is faster in woven compared to lamellar periosteal bone.

(c) *At 30 days*

Periosteal appositional bone in groups A, B and C presented a thick band of homogeneous densely labelled bone because remodelled lacunae had been filled by labelled concentric lamellae of new osteones (Fig. 3c).

(d) *At 90 days*

The periosteal activity of groups A and B did not differ from group C.

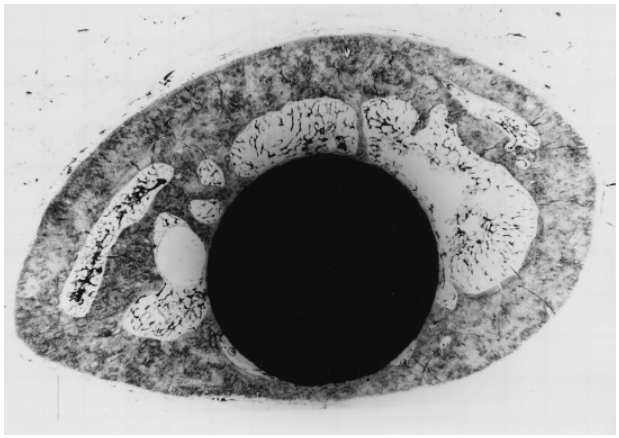

Fig. 5. Reamed cementless implant (group B). At 90 days, most of the endosteal reactive bone has remodelled, the implant is connected to the endosteal surface by bony bridges and medullary vascularisation is restored around it (×10, HE, vascular tree injected)

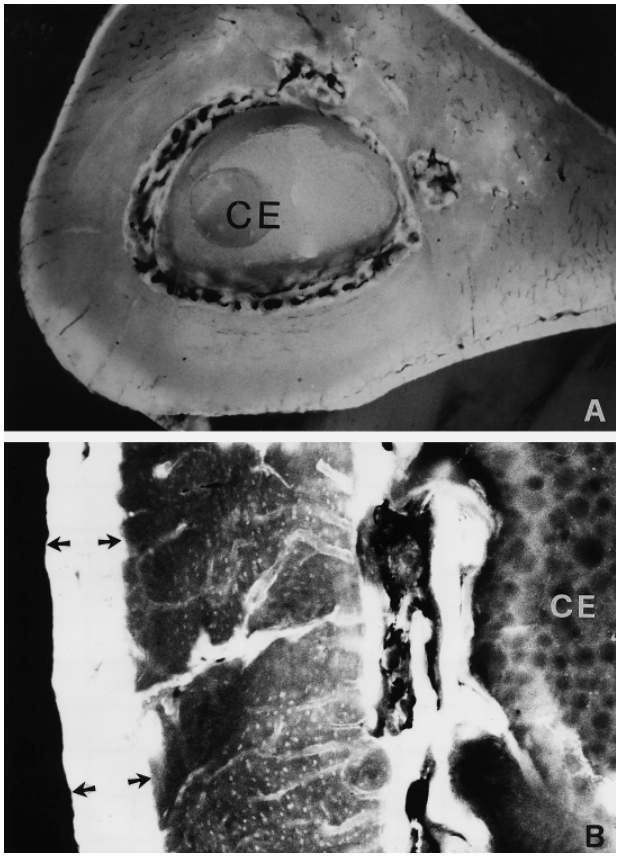

Fig. 6 A, B. Reamed cemented femur (group A). **A** At 30 days, new vessels penetrate the subendosteal area near the cement (CE = bone cement) (×12, fluorescence). **B** Remodelling of endosteal bone corresponding with the cement. *Arrows* indicate a band of labelled periosteal apposition (×80, fluorescence)

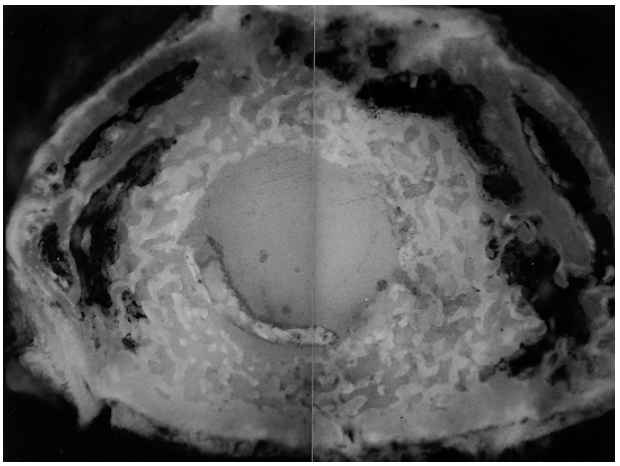

Fig. 7. Reamed cemented femur (group A). At 90 days, revascularisation of the cancellous metaphysis has demarcated the cement, incorporating necrotic trabeculae which are engulfed in the cement (×12, fluorescence)

Endosteal reaction of implanted femurs (groups A and B) and control reamed femurs (group C)

(a) *At 7, 15 and 30 days*

Endosteal reactive woven bone was present in both group C and group B, labelled woven bone filling the medullary canal or the space between the endosteal surface and the implanted cementless stem (Fig. 4).

(b) *At 90 days*

Group B showed remodelling of primary endosteal bone in the form of a thin lamellar capsule connected to the surface by a few radial bridges of differing consistency. A well developed network of injected vessels was present between the endosteum and the implant (Fig. 5).

Reactive endosteal bone had completely resorbed in group C.

Reaction in cemented femurs (group A)

(a) *At 7 days*

There was no reactive endosteal bone.

(b) *At 15 and 30 days*

Injected vessels had appeared near the bone-cement interface and signs of subendosteal remodelling were more obvious at 30 days.

(c) *At 90 days*

Extensive revascularisation of the subendosteal cortex had occurred. As a consequence of vascular lacunar formation, the mass of cement was in contact with a porous layer of bone rather than solid cortex. The connection between the cement and bone was through unresorbed cortical bone and not remodelled trabeculae (Fig. 6a, b). At the level of the metaphysis, the cement was demarcated by revascularisation incorporating old trabeculae (Fig. 7).

There was no sign of cortical remodelling at 7 days in any of the groups. Occasionally, labelled osteones were seen in the subperiosteal cortex in groups A and B at 15 days. Extensive remodelling involving the whole cortex was present in both these groups at 30 days.

Discussion

Our experimental model reproduces the insertion of a cemented and a cementless stem as used in orthopaedic surgery. The miniaturised technique was satisfactory and completely filled the canal with acrylic cement with good penetration of trabecular bone. Bone metabolism is more rapid in rats compared with man, but we consider it justifiable to assume that the changes are similar to those occurring in patients in whom cemented or cementless prosthesis are inserted, and when load bearing is avoided. The periosteal and endosteal reaction, and the revascularisation seen after reaming, have been recorded in fractures of human long bones and do not differ quantitatively from what is seen in the experimental model [18, 26]. However, what occurs in rats in 90 days would take several years in man.

The first response of rapid periosteal apposition appeared to be a general reaction to reaming rather than to cement or nailing since it is present to the same degree in the control reamed femur (group C). Within 2 weeks this activity ceased and the only change was remodelling of new bone. Although the width of the fluorescent band increased between 7 and 15 days, this represented calcification of already deposited bone, so the periosteal reaction only occured in the first 7 days.

The irregular distribution of fluorescence in reamed groups A and B shows a circumferential lamellar pattern of appositional bone, in contrast with woven primary bone in the unreamed group D where osteoblasts lay down collagen fibres without any spatial orientation. Apposition is also faster in groups C and D as shown by the difference in width of the fluorescent band, and the most recently formed matrix is not calcified or labelled.

Since the periosteum is not directly damaged by reaming, its response must be related to the changed pattern of vascularisation [4, 5]. Normally, the flow is centrifugal; two-thirds or more of its blood supply comes from the medullary vessels, and only the outer layer is supplied by periosteal vessels [20]. Reaming destroys the medullary vessels and the flow is temporarily reversed [17] with proliferation of the periosteal vessels. Injected vessels are also found in the middle of the cortex of cemented femurs which suggests that the flow is centripetal. The enlargement of the periosteal vessels is associated with an increase of osteoblasts with rapid production of woven bone during the first week. The bone is remodelled to osteonic lamellar type with an increase in the circumference of the femur. This contradicts Grundes and Reikeras who did not observe this increase in circumference, perhaps due to insufficient reaming which they describe as ‘modest’ [9].

Remodelling of the old cortex takes place at about 30 days and is mainly subendosteal, increasing the inner circumference and depending on revascularisation by the medullary vessels. Both factors affect fixation of the implant because the fit can loosen as the diameters increase.

In the conditions of our study, every change in the bone, whether apposition or remodelling is preceded by proliferation, progression and orientation of the vessels. Mechanical stability, conditions of loading, surface porosity and chemistry are thought to affect anchorage of implants to bone, but the pattern of blood supply, which we consider relevant in determining the organisation of lamellar bone around the implant, has so far been neglected.

Endosteal apposition is nonspecific reaction to reaming since it was present in both the implanted cementless group (B) and the control reamed group (C). It does not occur in cemented femurs possibly because the entire canal is occupied by acrylic so that stability is by interlocking of cement with the irregular bony surface. This is greatest soon after implantation and decreases as remodelling of the inner cortex with medullary revascularisation occurs [6, 10]. The process is far advanced in rats after 90 days, but is much slower in man where in retrieved stable cemented stems interlocking is seen to be reduced to a few points and most contact is with the connective tissue which has replaced bone. This occurs in the ab-

sence of foreign body reaction which would hasten the progressive reduction of the bonding area [15].

In cementless stems, the endosteal primary woven bone leads to ingrowth on the surface of the implant [1, 22], and remodelling forms a lamellar capsule connected by radial bridges to the endosteal surface [16]. The initial stability is assured by the primary fit of the implant, and evolution of the capsule increases fixation related to the number and thickness of bony bridges. Remodelling also occurred in this group (B), but has less influence on the stability of the implant.

References

1. Bobyn JD, Engh CA (1985) Biological fixation in total hip arthroplasties. Slack Inc., Thorofare NJ
2. Bragdon JA, Foster L, Sosman MC (1949) Experimental Infarction of Bone and Bone Marrow. Am J Pathol 25: 709–723
3. Brookes M, Harrison RG (1957) The vascularization of the rabbit femur and tibia-fibula. J Anat 91: 61–72
4. Brookes M (1971) The blood supply of bone. Butterworths, London, 1–338
5. Danckwardt-Lillienstrom G (1968) Reaming of the medullary cavity and its effect on diaphyseal bone. Acta Orthop Scand [Suppl] 128: 1–153
6. Danckwardt-Lillienstrom G, Lorenzi GL, Olerud S (1970) Intracortical circulation after intramedullary reaming with reduction of pressure in the medullary cavity. A microangiographic study in the rabbit tibia. J Bone Joint Surg [Am] 52: 1390–1394
7. Draenert K (1981) Histomorphology of the bone-to-cement interface: remodelling of the cortex and revascularization of the medullary canal in animal experiments. In: Salvati EA (ed) The hip proceedings of the ninth open Scientific Meeting of the hip society. Mosby, St Louis Toronto London
8. Feith R (1975) Side-effects of acrylic cement impianted into bone. Acta Orthop Scand [Suppl] 161: 2–136
9. Gothmann L (1960) The normal arterial pattern of the rabbit tibia after the application of an intramedullary nail. A microangiographic study. Acta Chir Scand 120: 201–210
10. Grundes O, Reikeras O (1994) Nailing and occlusion of the medullary cavity. Flow and mechanical changes in rat femura. Acta Orthop Scand 65: 175–178
11. Gustilo RB, Nelson GE, Hamel A, Moe JH (1964) The effect of intramedullary nailing on the blood supply of the diaphysis of long bones in mature dogs. J Bone Joint Surg [Am] 46: 1362–1363
12. Harms J, Van De Berg PA, Mertz L (1974) Knochen-revascularization nach Mefobaine-Polacos-Fullung. Eine experimentelle untersuchung an der hund tibia. Arch Orthop Unfall-Chir 80: 71–78
13. Koekenberg LJL (1963) Vascularization in the healing of fractures. Thomas, Springfield
14. Nelson GG, Kelly PJ, Lowell FA, Peterson LFA, James JM (1961) Blood supply of the human tibia. J Bone Joint Surg [Am] 42: 625–635
15. Oni OOA, Stafford H, Gregg PJ (1987) An investigation of the routes of venous escape from the diaphyseal marrow of canine and rabbit long bones. Int J Microcirc Clin Exp 7: 31–41
16. Pazzaglia UE (1990) Pathology of the bone-cement interface in loosening of total hip repleacement. Arch Ortop Traum Surg 108: 83–88
17. Pazzaglia UE, Zatti G, Cherubino P, Frick W, Koch R (1991) Bone reaction to the implant of intramedullary pins of three different metals in the rats femur. Mater Med 2: 77–81
18. Reichert ILH, Mc Carthy ID, Hughes SPF (1995) The acute vascular response to intramedullary reaming. Microsphere estimation of blood flow in the intact ovine tibia. J Bone Joint Surg [Br] 77: 490–493
19. Richany SF, Sprinz H, Kramer K, Ashby J, Mevrill TG (1965) The role of the diaphyseal medulla in the repair and regeneration of the femoral shaft in the adult cat. J Bone Joint Surg [Am] 47: 1565–1584
20. Rhinelander FW, Granilla RW, Phillips RS, Steel WM (1967) Microangigraphy in bone healing III. Osteotomies with internal fixation. J Bone Joint Surg [Am] 49: 1006–1009
21. Rhinelander FW (1968) The normal microcirculation of diaphyseal cortex and its response to fracture. J Bone Joint Surg [Am] 50: 1273–1298
22. Rhinelander FW, Nelson CL, Stewart RD, Steward CL (1979) Experimental reaming of the proximal femur and acrylic cement implantation. Clin Orthop 141: 74–89
23. Schenk RK, Herrmann W (1984) Histologic studies on the incorporation of uncemented implants. Cementless fixation of the hip endoprostheses. In: Morscher E (ed), Springer, Heidelberg, pp 52–58
24. Shim SS, Copp DH, Patterson FP (1968) Measurement of the rate and distribution of the nutrient and other arterial blood supply in long bones of the rabbit. A study of the relative contribution of the three arterial systems. J Bone Joint Surg [Br] 50: 178–183
25. Sund G, Rosenquist J (1983) Morphological changes in bone following intramedullary implantation of methylmetacrylate. Effects of medullary occlusion: a morphometrical study. Acta Orthop Scand 54: 148–156
26. Trias A, Fery A (1979) Cortical circulation of long bones. J Bone Joint Surg [Am] 61: 1052–1059
27. Trueta J, Cavadias AX (1955) Vascular changes caused by the Kuntscher type of nailing. J Bone Joint Surg [Br] 37: 492–505
28. Trueta J (1963) The role of the vessels in osteogenesis. J Bone Joint Surg [Br] 45: 402–418