ACTA ORTHOPAEDICA SCANDINAVICA
Supplementum No. 161

From the Laboratory for Experimental Orthopaedics, Department of Orthopaedics (head: Prof. Dr. Th. J. G. van Rens), Catholic University, Nijmegen, The Netherlands.

SIDE-EFFECTS OF ACRYLIC CEMENT IMPLANTED INTO BONE

A HISTOLOGICAL, (MICRO)ANGIOGRAPHIC, FLUORESCENCE-MICROSCOPIC AND AUTORADIOGRAPHIC STUDY IN THE RABBIT FEMUR

BY

R. FEITH

MUNKSGAARD - COPENHAGEN 1975

Translated by: Th. van Winsen

The publication of this study was financially supported by grants from the
Jan Dekkerstichting and Dr. Ludgardine Bouwmanstichting
and Hippocrates Studiefonds.

ISBN 87 16 02156 8

Printed in The Netherlands by
Drukkerij Brakkenstein, Nijmegen, 1975

To Annelies
and to the memory of
my first and finest teacher,
my grandfather
Jonkheer J. M. de Jonge, C.E.

ACKNOWLEDGEMENTS

To do experimental work in addition to normal clinical daily duties is made possible only by the know-how, help and positive criticism made available by other workers, scientific or otherwise. All who in this way have contributed to the completion of this thesis receive my heartfelt thanks.

I wish to express my gratitude to Professor Dr. Th. J. G. van Rens for making available time and for his interest throughout this study. The incentive to this investigation was given by Dr. T. J. J. H. Slooff and I have greatly appreciated his dedicated support throughout the experiments and their analysis.

In particular I have made grateful use of the actual help and advice of J. R. de Wijn Chem. E. (Department of Dental Materials), Dr F. W. Rhinelander (Professor of Orthopaedic Research, University of Arkansas, Little Rock, USA), R. A. M. J. Claessens Chem. D. (Department of Nuclear Medicine), J. R. Bakker, dispensing chemist and J. Ellul, anesthesiologist.

The original ideas and helpfulness of Messrs. T. J. M. Arnoldussen (Instrument Workshop), A. J. Peters, Th. H. M. Arts, P. H. G. Philipsen and G. J. T. Grutters (Central Animal Laboratory) have been of much importance to me.

Mrs Mieke H. J. Jacobs-Van den Hombergh and later Miss Diny H. J. Versleyen and Miss Gemma M. J. Bongers (Laboratory for Experimental Orthopaedics) prepared the specimens, and the autoradiograms were made with the technical assistance of N. V. M. Rijntjes (Dispensary). Indispensable assistance was given by F. E. Schrijer (Instrumental Service) in fluorescence photomicrography. The drawings were supplied by the department of Medical Illustration (head: J. J. M. de Bekker). I have greatly appreciated the efforts of the department of Medical Photography (head: A. T. A. Reynen), which supplied the photomicrographs and advice in the lay-out of this thesis. The manuscript was typed with great care by Miss Irene A. Keizer and Miss Petra M. G. Jansen.

CONTENTS

INTRODUCTION

The range of application of polymethylmethacrylate (PMMA) as acrylic bone cement in orthopaedic surgery is steadily increasing. In particular, the use of acrylic cement has facilitated the solution of numerous problems in the fixation of artificial joints. This has made it possible to expand the use of artificial joints and to give many patients with invalidating arthropathies a better life.

According to Müller (1974b), some one-million patients have so far been treated by total hip replacement in Europe and the USA; and Bloch (1974) estimates that about a thousand total hip arthroplasties are now being performed every day all over the world!

Since the first total hip replacements with acrylic cement fixation were performed in the early Sixties, continued research has focused in particular on improvements in the mechanical characteristics of metal and plastic prosthetic components. The orthopaedic surgeon is confronted with an unceasing flow of new types of prostheses, but acrylic bone cement has shown no fundamental modifications or improvements since it was first used by Charnley (1960). This is perhaps explained by the fact that the use of similar products in dentistry had been preceded by a long period of research.

Although acrylic cement has generally lived up to its expectations, problems of fixation have not yet been fully solved. Loosening of the prosthesis is being encountered in an increasing number of total hip replacements, often years after its implantation and after an initial clinical course which could only be described as favourable (Amstutz 1970; Wilson and Scales 1970; Wilson et al. 1972; Patterson and Brown 1972; Salenius and Laurent 1973; van Rens 1973; Müller 1974a, 1974b; Deutman 1974; Dandy and Theodorou 1975). This late loosening, which totally invalidates a good result, is one of the greatest problems of total hip replacement today. According to Müller (1974b), a 10-year follow-up on patients treated by total hip replacement can be expected to show that some 20 per cent will need a secondary operation in view of late complications, loosening being the most common. This applies to patients operated on in highly specialized clinics by experienced surgeons.

It is not easy to diagnose „loose prosthesis", and it may be several years before the situation is clinically evident. Arthrography (Salvati et al. 1971) and Strontium ^{87m} Sr bone scanning (Feith et al. 1975) have proved to be

valuable diagnostic aids. The late complication rates reported, even in larger series, should not be taken for granted. It may well be that patients coming back with „obscure pain" and „diminished mobility and function" are really suffering from a loose prosthesis and in whom the diagnosis has been missed. The proper diagnosis should have increased the total percentage of „late loosening". Moreover, in cases of late infection (always associated with loosening of a prosthesis component) it is rarely possible to establish whether the aseptic loosening or bacterial infection was the primary development.

Possible causes of late loosening of normally used total hip prostheses include: necrosis and resorption of the bony implant bed, insufficient cementing, excessive friction between the components of the prosthesis, infection, shrinkage of acrylic cement and sensitization to detached metal particles resulting in low-grade infection. All these processes take place at the interface between bone and acrylic cement. Starting from the operation, the bone at this site is exposed to, successively, mechanical lesions caused in preparing for the implantation, and thermic as well as chemical-toxic influences exerted by the cement. Partly due to interruption of the bone vascularization and partly due to direct damage, this results in the thin layer of necrosis of the bony implant bed described by Charnley (1970) and by Willert and Puls (1972) and Willert et al. (1974b). As a result, the initial direct contact between bone and cement is partly lost. In the majority of cases adaptation of the implant by organization of the necrosis and regeneration of new bone is sufficient to produce what could be described as the recipient's tolerance to the foreign body. In other cases, however, in which the fixation of the components by the acrylic cement is lost, this necrosis might well be the principal cause of late loosening of the prosthesis; and it might also be the factor predisposing to late infection.

The reasons why this necrosis develops has so far remained obscure. The purpose of this study is to investigate the three factors generally accepted as causative, separately and in combination, in experiments using the rabbit femur. These factors, of which the first two should be regarded as local side effects produced by the acrylic cement, are:

1. the high maximal polymerization temperature of the acrylic cement setting in situ;
2. the local cytotoxic effect of the monomer;
3. interruption of the osseous vascularization by surgical manipulations required to prepare for implantation.

Part of this study was presented as preliminary report at the 7th International Biomaterials Symposium held in Clemson, USA (Feith and Slooff 1975).

CHAPTER 1

ACRYLIC BONE CEMENT

1.1. *Introduction*

Polymethylmethacrylate (PMMA) was first used on a larger scale in orthopaedic surgery when the brothers Robert and Jean Judet introduced their Perspex or Plexiglas femoral head prosthesis in 1946. The initial results seemed promising (J. Judet et al. 1952) but in the longer run the acrylic prosthesis was found to be biologically and mechanically unsuitable for clinical use (Müller 1962; Anderson et al. 1964; Salvati and Wilson 1973). The Judet type metal prosthesis (La Chapelle) was likewise found to be unsatisfactory. The subsequently developed metal head-neck prostheses (best-known types: Austin-Moore, Thompson, Eicher and Müller) were a marked improvement. However, not all biomechanical problems were yet solved. The fixation of these hemiarthroplasties remained the principal problem.

The interest in PMMA, but now as „bone cement", was revived when Charnley (1960) – advised in his choice of plastic by D. C. Smith – stabilized his first hemiarthroplasty of the hip with cold-curing PMMA. Shortly after, Charnley (1961) and McKee and Farrar (1966) each developed a total hip prosthesis which they anchored with acrylic cement. Since the early Seventies, this bone cement has become an indispensable aid in orthopaedic surgery. The method of fixation which fills the space between the joint replacement and previously prepared bone defects with malleable plastic setting in situ, was revolutionary. It was therefore not until several years later that the concept of relying on the strength of the cement to support the prosthesis rather than having it rest on the bone, was generally accepted. Acrylic cement heralded the era of total joint replacements.

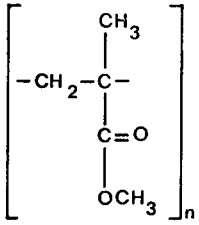
Excellent studies on PMMA have since been published in Dutch (Slooff 1970), English (1970) and German literature (Contzen et al. 1967; Oest et al. 1975*). To avoid repetition, the following sections will review only such aspects as are relevant to this study, and will discuss some recent developments.

* Published after completion of this manuscript.

1.2. *General characteristics*

1.2.1. Composition of the components

Polymethylmethacrylate is a plastic material composed of macromolecules. These in turn are made up of smaller units called monomers which, linked by chemical double bonds as a result of polymerization, form the links of the polymer chain. The everrepeated unit in acrylic bone cement is the methyl ester of methacrylic acid (methylmethacrylate). Therefore the chemical name of the polymer is polymethylmethacrylate:



The material is prepared for implantation by mixing polymer powder with liquid monomer, which consists mainly of methylmethacrylate. The powder consists of tiny spherical particles of polymerized polymethylmethacrylate to which about 1 per cent benzoyl peroxide has been added (the initiator or catalyser).

The powder can in addition contain co-polymers of methylmethacrylate with butylmethacrylate and styrene. It is also possible to add some radiopaque substance such as barium sulphate or zirconium dioxide, as well as antibiotics. The latter are released in a locally effective dose (Wahlig et al. 1972; Wahlig and Buchholz 1973; Koschmieder et al. 1973, 1975). The highest effective local concentration can be attained with gentamycin sulphate (Refobacin®, Garamycin®, usual dosage 500 mg/40 mg polymer powder) (Hessert and Ruckdeschel 1970; Buchholz and Gartmann 1972; Wahlig et al. 1972; Ruckdeschel et al. 1973; Marks et al. 1975).

After release from the cement of initially high microbiological concentrations of the antibiotic during the first three days, it is believed that a minimum inhibitory concentration of sufficient height can be maintained in the immediate vicinity of the implant up to 5-6 months after implantation (Wahlig et al. 1972; Wahlig and Buchholz 1973). According to Buchholz (1973), the antibiotic is still demonstrable even after 24 months. It is believed that addition of 100 mg streptomycin to the polymer powder ensures sufficiently high tuberculostatic concentrations in the tissue surrounding the implant (Knapmann 1974).

The liquid monomer is an inflammable, volatile, highly lipophilic fluid which has been demonstrated to be cytotoxic (Hulliger 1962; Willert et al. 1973c). A stabilizer or inhibitor (hydroquinone) has been added to it in order to prevent spontaneous polymerization; as activator has been

added a tertiary amine (dimethylparatoluidine), which releases from the benzoyl peroxide the free radicals required to open the double carbon bond in the monomer. No heat is required to initiate the reaction; hence the term cold-curing.

1.2.2. Handling and curing characteristics

The components form a system in which the relative quantities have been carefully determined by the manufacturer, and it is ill-advised for the surgeon to alter the proportions indicated. The mixing of polymer powder and monomer liquid produces a soft paste which has the typical odour of the volatile monomer. In order to ensure maximum escape of the cytotoxic monomer prior to implantation, the substance is not introduced until after 1.5-4 minutes, when it has become putty-like and there is just sufficient working time left to mould it at the appropriate site in the bone, whereupon the prosthesis is placed in position. It is of importance to ensure that the cement mass is still sufficiently malleable to enter even the smallest interstices in the bone. After waiting too long, the cement laminates and becomes mechanically weaker (Lee et al. 1973).

Kutzner et al. (1974a) determined by biological means that, after mixing acrylic cement (Palacos) at normal speed for 2.5 minutes, a total of 1.11 per cent monomer is released by evaporation up to 20 minutes after joining the components. Lee et al. (1973, 1975) found that monomer evaporation is promoted by thorough mixing; especially important was their finding that the amount of monomer released is directly proportional to the rate of stirring. Up to 14 per cent of the monomer could evaporate at a stirring rate of 260 beats per minute. The duration of mixing also played a role, but was less important. When mixing was discontinued, monomer evaporation was slight; this was confirmed by Roggatz (1974) and Debrunner and Wettstein (1975b). When mixing was resumed for 1 minute, ten times as much monomer was found to evaporate as when the cement was left alone. A similar effect was obtained by manual kneading for 1 minute (Lee et al. 1973, 1975). It is to be pointed out that manual kneading accelerates the setting process and slightly increases the maximal temperature peak due to an increase in ambient temperature (Haas et al. 1975).

Willert et al. (1973c) demonstrated in an experimental study that the cytotoxic effect of escaping monomer can be substantially reduced by having polymerization take place outside the body as long as possible. In a preliminary report, Debrunner and Wettstein (1975b) maintained that the principal monomer release occurs during the increase in temperature at polymerization. In their arrangement, no release was measurable any longer 2-3 minutes after setting.

The exothermic polymerization process needs some time to get properly started. With increasing heat release the phase in which the material is plastic ends, and setting commences (fig. 1). When the cement is setting, the maximum curing temperature is reached at about the same time; this can locally be as high as about 100° C. The setting process still continues,

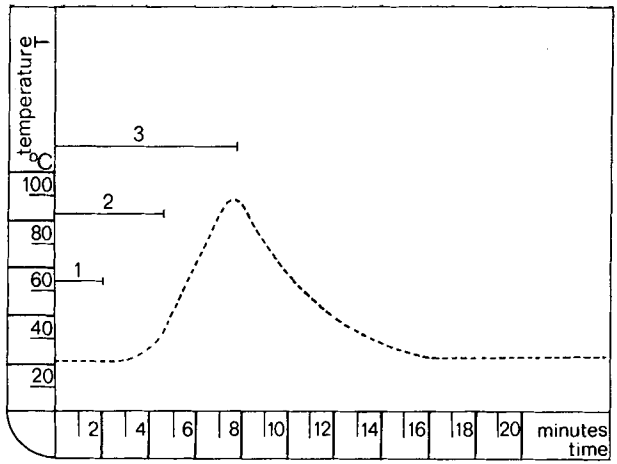


Fig. 1: General shape of a temperature-time curve of acrylic cement; to elucidate the concepts of terms mixing time (1), working time (2) and setting time (3) these are represented schematically. The duration indicated for (1), (2) and (3) is arbitrary.

and it is only after several hours that the material attains its definitive degree of hardness (Haas et al. 1975).

The working time of the various commercial bone cements varies considerably (Willert et al. 1973c; de Wijn et al. 1975; Debrunner and Wettstein 1975a). Other factors also play a role. For example, when the powder : liquid ratio diminishes (thereby increasing the amount of monomer), the setting time increases and the polymerization temperature attains a higher maximum (Meyer et al. 1973).

Apart from the constituents, the speed of the reaction is also dependent on the volume of the cement mixture and on the ambient temperature. The larger the cement mass, the longer the setting time. Not only the room temperature but also that of mixingbowl, spatula, operation lamp, and the body temperatures of surgeon and patient must be taken into consideration (Meyer et al. 1973). A higher ambient temperature shortens the working time and setting time, and vice versa. By increasing the ambient temperature by 10° C, the polymerization process is accelerated by factor 1.5-2; and the working time is therefore halved (Debrunner and Wettstein 1975a). The maximum polymerization temperature attained also increases as the ambient temperature rises (Meyer et al. 1973).

1.2.3. Polymerization heat

It has been reported that about 130 calories are released in the reaction per gramme of polymerizing monomer (Meyer et al. 1973; Labitzke and

Paulus 1974). This implies that the total amount of heat produced increases when a larger amount of acrylic cement is used, while the maximum polymerization temperature increases as well. As the polymerization process advances, the reaction accelerates and more calories are released per unit of time (Debrunner 1974). The maximally attainable polymerization temperature is variously reported in literature as from 40 to 120° C (Wiltse et al. 1957; Slooff 1970; Debrunner 1974). The wide range of variation in laboratory results is explained by Slooff (1970) and by Hupfauer and Ulatowski (1971) on the basis of insufficient standardization of:

1. the measuring devices used;
2. the site of measurement in the cement;
3. the amount and consistency of the cement;
4. the monomer : polymer mixing ratio;
5. the surrounding material;
6. possible supply and release of heat;
7. measuring in vitro or in vivo.

Of course the maximum temperature of the cement surface measured in vivo is conclusive of any possible thermic tissue damage. In this context the German-speaking authors attach great value to the critical limit of 56° C indicated by Lehnartz (1959), at which body protein is alleged to coagulate, or to a limit of 70-72° C which Labitzke and Paulus (1974) hold to be the temperature at which bone collagen coagulates. These authors maintain that thermic necrosis need not be feared as long as one remains below this limit.

In in-vivo measurements in the bone/cement interface in dogs, Homsy et al. (1972a) found temperatures of 70-90° C. Meyer et al. (1973) reported having found a maximum temperature of 70° C in the bone/acrylic interface during total hip arthroplasty in 10 different patients. In 10 patients treated by total hip arthroplasty, Biehl et al. (1974) measured in the trochanteric region a mean temperature of 46.6° C (range 42.6-51° C), and Labitzke and Paulus (1974) reported a mean temperature of 45.2° C (± 4° C) in 5 such patients. The lastmentioned authors found a mean maximum temperature of 50.4° C (± 7° C) at the interface of the prosthesis cup. Biehl et al. (1974) measured a mean temperature of 43.6° C (range 43-45.9° C) at the end of the femoral component of the prosthesis. Near the acrylic cement, and according to Debrunner (1974) from only 3 mm from the cement surface on, the maximum measurable temperature is much less high: not exceeding two-fifths of the temperature of the cement surface. He thought it likely that in living tissue with an intact blood circulation the increase in temperature would be even less

than in the model set-up. This hypothesis was confirmed by Biehl et al. (1974) in comparative measurements at the interface between bone and cement during implantation of total hip and knee replacements. The knee prostheses were introduced under tourniquet ischaemia, and in these cases unmistakably higher temperatures were measured (on average 9.4° C higher).

Irrigation of bone surface and prosthesis has no significant effect on the maximum temperature (Ohnsorge and Goebbel 1970; Meyer et al. 1973). It was found possible to reduce the maximum polymerization temperature by using prostheses cooled to 0° C (Ohnsorge and Goebbel 1970), by adding metal powder to the cement mixture (Homsy et al. 1972), and by addition of co-polymers with longer chains (de Wijn 1974). The lastmentioned principle was used in the manufacture of the commercial acrylic cement Sulfix®, discussed in section 3.2. It is doubtful, however, whether these methods can sufficiently reduce the temperature to prevent thermic tissue damage. Moreover, the use of cooled prostheses has a number of disadvantages. For example, the marked auto-accelerating effect of the exothermic reaction, which gives this cement such attractive features for use in fixation, is inhibited (de Wijn 1974). Secondly, insufficient polymerization has an unfavourable effect on the mechanical properties of acrylic bone cement, and increases the percentage of monomer left in the cement (Puhl and Schulitz 1971; Willert et al. 1973c).

1.2.4. Mechanical properties

Studies with the scanning electron microscope (SEM) have shown that the structure of acrylic cement consists of an aggregate of previously polymerized polymer pearls, retained by cohesion to recently polymerized monomer. Polymerized PMMA, therefore, is a composite material in which no chemical bond has been established between polymer pellets and the monomer methylmethacrylate.

Many investigators have submitted the material to mechanical tests, demonstrating that it offers great resistance to compressive stress, but less resistance to tensile and shear stresses (Wiltse et al. 1957; Charnley 1970; Lautenschlager et al. 1974; de Wijn et al. 1975b; Haas et al. 1975).

Several factors exert a negative influence on the mechanical properties.

1. Acrylic cement set in situ shows a certain degree of porosity due to inclusion of air bubbles during mixing and evaporation of monomer during the marked increase in temperature. The pores form a closed system and therefore do not communicate with each other. Porosity and mechanical hardness are inversely proportional (Debrunner 1975; de Wijn et al. 1975b).

2. Apart from air bubbles, acrylic cement setting in situ also loses strength due to admixture of blood and bone marrow. The manner of application of the cement also influences its homogeneity. Apart from other advantages of using a cement syringe, the risk of gross irregularities in the cement mass and a difference in consistency between the first and the last cement plug is much less after introduction by means of a syringe than after digital introduction (Slooff 1969; de Wijn et al. 1974a).
3. Addition of a radiopaque substance and/or an antibiotic was reported not to interfere with the mechanical strength of the cement (Hessert 1971; Marks et al. 1975). This, however, was refuted by Grünert and Ritter (1974), de Wijn et al. (1975b) and Haas et al. (1975), who demonstrated that these additions cause mechanical weakening of the cement due to loss of homogeneity and increased water absorption.
4. Finally, there are indications that acrylic cement ages in situ. Water absorption alters the mechanical quality (Bloch and Hastings 1972; Wagner and Bourgeois 1974).

Oest and Müller (1973) reported that test specimens of acrylic cement polymerized under pressure (dimensions: 60 x 6 x 4 mm) and when kept in Ringer solution at 20°C, were found to be saturated with water after about 75 days. Their weight had increased by 1.6 per cent, and they had therefore lost 20 per cent of their hardness. An increase in temperature to 40°C resulted in another 20 per cent loss of hardness (Oest and Müller 1973). Jaffe et al. (1974), however, were unable to demonstrate any significant deterioration of static properties or compression fatigue behaviour in specimens of acrylic cement kept in bovine serum at 37°C for up to two years.

As a result of the above-mentioned factors the ultimate compressive and tensile strengths of Simplex-P set in vivo are, according to Homsy (1973) and Lautenschlager et al. (1974), on average only 20-35 per cent of those of polymer obtained under factory conditions. Acrylic cement obtained at re-operation was submitted to various strength determinations and found to be 50 per cent weaker than homogeneous test specimens (Oest and Müller 1973; de Wijn et al. 1974a, 1975b).

de Wijn et al. (1974a, 1975b) reported that in particular the greater porosity of PMMA setting in situ has an unfavourable effect on its mechanical properties. It is difficult to answer the question whether weakening of the acrylic due to the above-mentioned factors is of essential significance for the success or failure of a joint replacement operation. No exact figures are available on the minimum requirements which the mechanical properties of acrylic cement should meet (de Wijn et al. 1975b).

Acrylic cement is not a glue, nor does it form any bond with living tissue at the molecular level. It is exclusively a filler, which mechanically

stabilizes the cemented prosthesis. Compressive, torsional and tensile forces are divided over a large bone surface area by the cement (Charnley 1970). Anchoring is further enhanced by intimate interlocking of the cement with surface irregularities.

Stabilization of a prosthesis with acrylic cement partly depends on the number and the efficiency of interlockings of projections of the cement surface with surface irregularities of the surrounding bone tissue (Homsy 1973). The layer of fibrous connective tissue which forms at the interface (cf. pages 25 to 30) allows minimal movements between bone and cement surface. Charnley (1965, 1970) does not regard these as a disadvantage: in fact he rather regards this fibrous interposition as a shock absorber which permits of elastic deformation between bone and cement – substances of different stiffness (the stiffness of PMMA is less than that of cortical bone but exceeds that of cancellous bone).

Mechanical theories become substantially more complex when the mechanical strength of the in-vivo „bond" between bone and acrylic cement is to be calculated, with the prosthesis in situ or otherwise. Numerous variables are then to be taken into account, e.g. body weight, jolting stress, vascularization, mineralization and architecture of the bone as well as position, shape and mechanical properties of the prosthesis (Charnley 1965, 1970; Slooff 1970; Wilson and Scales 1970; Kölbel and Boenick 1972; Oest 1973; Kölbel et al. 1973; Ritter et al. 1973; Walker 1973; Jäger et al. 1974; Chen et al. 1974). This general introduction does not lend itself to a detailed discussion of this complex subject.

1.2.5. Volumetric changes

Theoretically predictable shrinkage (Contzen et al. 1967; Haas et al. 1975) of the product of polymerization was not corroborated by Charnley (1970). He measured an increase in volume by 3-5 per cent. In subsequent studies Hupfauer (1973), Ohnsorge and Grötz (1974), Debrunner (1975), de Wijn et al. (1975c) and Haas et al. (1975) concluded that, after a slight initial increase in volume due to thermic expansion and foaming of the mixture, cooling of the mass to ambient temperature after curing causes a decrease in volume. The percentages of decrease in volume reported by the above-mentioned authors vary from 1-4 volume per cent depending on the technique used. In other words: the cement shrinks during the period of rapid polymerization. As the cement mass absorbs water, some of this volume loss can be regained in the long run (Oest and Müller 1973; Ohnsorge and Grötz 1974; de Wijn et al. 1975c; Haas et al. 1975).

Whether this slight decrease in volume during the final rapid phase of polymerization might contribute to loosening of prostheses is unknown. In any case, this inevitable shrinkage cannot be expected to enhance fixation.

1.2.6. General toxicity of the monomer

The question of the general pharmacological effect of monomer on the human organism has received renewed attention since 1970. The reason was that cardiovascular complications were an unpleasant surprise in a procedure (total hip arthroplasty) which was rapidly gaining acceptance. Apart from reports on transient hypotension, usually observed immediately after introduction of acrylic cement and prosthesis into the femoral shaft, the Anglo-American literature in particular presented from 1970 onwards a large number of case reports describing peroperative, sometimes irreversible cardiac arrest during hemiarthroplasty and total arthroplasty of the hip and total knee replacement. Series of such case reports recently compiled by Milne (1973), Dandy (1974) and Herndon et al. (1974) can probably be supplemented with a by no means negligible number of unpublished cases. Despite Charnley's denial (1970) and its corroboration by the anaesthetists of the Wrightington Hospital (Brittain and Ryan 1972), some authors maintained that these cardiovascular complications were to be largely ascribed to the cytotoxic, lipophilic (residual) monomer. Later research has differentiated these views.

Once PMMA is set, 2-5 per cent residual monomer remains in the cement and 1-2 per cent is gradually diffused to enter the tissues in minute amounts (Smith and Baines 1956; Kutzner et al. 1974a; Haas et al. 1975). According to Hullinger (1962) the initiator, activator and inhibitor are not cytotoxic in the small quantities normally used.

A discussion of the local cellular and acute pharmacological cytotoxicity chiefly refers to the non-polymerizing monomer which is released in the organism during the reactive process. The possibly unfavourable effect of the so-called residual monomer (2-5 per cent) is negligible. The only thing really known about the local deleterious effect of non-polymerizing monomer is that methylmethacrylate is cytotoxic. The extent to which it may possibly contribute to tissue necrosis is unknown. No research in this direction has so far been undertaken.

According to Kutzner et al. (1974a), methylmethacrylate is pharmacologically active in 1.11 per cent of the monomer added to the mixture (page 15). Monomer can be demonstrated in venous blood after intrafemoral application of acrylic cement in dogs (Homsy et al. 1972a; McLaughlin et al. 1973) and human subjects (Bloch et al. 1970; Kim and Ritter 1972; Philips et al. 1973; Pahuja et al. 1974). The maximum concentration is reached during the rapid increase in polymerization temperature, but shortly afterwards it is no longer demonstrable in venous blood samples. Intravenously administered to test animals, the monomer was found to have three pharmacological effects:

a. It increased the rate of respiration, and in increased doses led

to respiratory arrest by a direct inhibitory effect on the respiratory centre (McLaughlin et al. 1973; Kutzner et al. 1974b);

b. It caused hypotension, probably as a result of decreased peripheral resistance in spite of an increase in cardiac output which as such might be caused by an increasing heart rate (Homsy et al. 1972a; Peebles et al. 1972; Holland et al. 1973; McMaster et al. 1974; Ellis and Mulvein 1974; Berman et al. 1974);

c. In large doses, it caused degeneration of pulmonary, hepatic and renal parenchyma (Contzen et al. 1967; Homsy et al. 1972a; McLaughlin et al. 1973; Holland et al. 1973).

The effects on respiration and blood pressure were both dependent on the monomer dose given.

The hypotensive effect, moreover, can apparently be potentiated by a deficiency in circulating volume. In addition, Berman et al. (1974) demonstrated in hypovolaemic dogs a decrease in cardiac output which in their view was caused by venodilatation (monomer effect), resulting in venous pooling and diminished venous return. It may also be mentioned that no ECG changes were found in test animals.

In an analysis of possible local and systemic side effects of acrylic cement in human individuals, Convery et al. (1975) were unable to demonstrate any untoward effects of PMMA on pulmonary, hepatic and renal function in patients after total hip replacement. The majority showed mild transient hypotension, especially during application of acrylic cement and insertion of the endoprosthesis into the femoral shaft. In the 10 patients whose cardiovascular function was recorded during the operation, the most constant change was a decrease in mixed venous oxygen saturation. The most logical explanation in their view was a decreased cardiac output, probably caused primarily by peripheral venous pooling. The authors (Convery et al. 1975) apparently advanced their theory on the basis of Fick's principle; this, however, applies only in a steady state, and it is questionable whether a steady state is really maintained during introduction of acrylic cement and a prosthesis into the femoral shaft. It is difficult to be certain what possible effect the monomer on its own can have. So many other factors can influence the circulation.

Modig et al. (1973b) stated in a preliminary report that fat particles were found in blood samples from the pulmonary artery immediately after insertion of the femoral prosthesis in three out of four patients undergoing total hip arthroplasty. With the aid of a Doppler flowmeter placed over the femoral vein, Herndon et al. (1974) demonstrated fat embolism in patients during total hip replacement. The largest number of „chirps" were heard during insertion of the femoral prosthesis. Only an occasional chirp was heard during introduction of the cup, preparation

of the proximal femur and introduction of the cement into this. The fat emboli thus demonstrated were also identified in blood samples obtained from the femoral vein during the operation. In a painstaking postoperative investigation they found no indications of systemic fat embolism. The number of fat emboli could be unmistakably reduced by venting the medullary cavity with a catheter attached to suction during introduction of the prosthesis, or by making burr holes in the lateral femoral cortex.

Although Herndon et al. (1974) did not explicitly state this, these fat emboli can be assumed to have originated from the medullary cavity (chapter 2, page 45) in view of findings reported by Danckwardt-Lillieström et al. (1970a, 1970b) and Kallos et al. (1974). The emboli enter the blood stream as a result of increased intramedullary pressure during the operation, particularly while the femoral prosthesis is being forced into the cement mass. Pressure determinations in patients during total hip replacement have confirmed the relevant results of animal experiments (e.g. Philips et al 1973; Tronzo et al. 1974). It is quite conceivable that a causal relationship exists between these fat emboli and the above-mentioned acute peroperative cardiac arrests, for postmortems have repeatedly revealed massive pulmonary fat and bone marrow embolisms in these casualties. (Milne 1973; Dandy 1974; Herndon et al. 1974).

In these cases, however, external heart massage had been applied; and in view of observations made by Jackson and Greendyke (1965) and Zichner (1970) the above-mentioned findings should be viewed with some reticence, for these authors found fat and bone marrow emboli in the lungs at postmortems on 80 per cent of patients who had died after external heart massage without preceding trauma. Regarding the cardiovascular complications in these usually elderly patients, some relation to type and concentration of drugs administered during anaesthesia also seems likely, even though it is refuted by some authors (Brittain and Ryan 1972). Others (e.g. Fearn et al. 1972) have confirmed it. However, comparison of anaesthetic techniques is almost impossible.

To summarize the above, it can be stated that it has not so far been demonstrated with certainty that the monomer, in the amount released during such procedures as total hip replacement, has any general pharmacological side effects on human patients. Cardiovascular complications during hip replacement are more readily explained on the basis of systemic fat embolism. A possible hypothesis is that of a combined effect or even an interaction between monomer and fat embolism, in the sense that the monomer is partly responsible for the development of fat emboli. And the high polymerization temperature of the cement may also be of significance in this respect.

The only study referring to a combined effect has been one by Schlag et al. (1973), who studied various cardiovascular functions in rabbits and dogs during intrafemoral

application of acrylic cement. They advanced the hypothesis that circulatory decompensation results from an increased pulmonary vascular resistance, to which three factors contribute. Fat and bone marrow emboli are held responsible for mechanical obstruction of the pulmonary microcirculation, believed to be further aggravated by the release (potentiated by the bone marrow emboli) of a thromboplastin-like substance giving rise to platelet aggregates. The last suggestion was also made by Modig et al. (1973a). The third factor, directly attributed to the monomer, is described to produce oedema of the alveolocapillary membrane in the lungs, which via reduction of the pulmonary microcirculation will contribute to increased pulmonary vascular resistance which could end in acute cor pulmonale.

The possibility of interaction between monomer and fat embolism, on the other hand, was suggested in studies by Dustmann et al. (1972) and Koch et al. (1972). They demonstrated histological features of extensive pulmonary embolism in six out of eight cats submitted to subtrochanteric femoral osteotomy, reaming of the medullary cavity and intramedullary implantation of bone cement. When the cement application was preceded by pressure-relieving suction drainage, mild fat embolism was demonstrable in only five out of ten animals. Fat embolism was likewise only slight when a different substance (Plasteline) was introduced into the medullary cavity without suction drainage; this fat embolism became more marked when liquid monomer was introduced into the femoral shaft in addition. When the procedure was repeated, this time applying acrylic cement after intravenous injection of 5 ml of the fat emulsifier choline phospholipid (Lipostabil), only two of the ten animals showed slight pulmonary fat embolism. Of the ten animals in which the Lipostabil injection was combined with suction drainage of the medullary cavity, none developed pulmonary fat embolism. The authors concluded from these results that the monomer is of essential significance in the causation of fat embolism.

In conclusion, it can be stated that even though no definite results have been reached, it can safely be admitted that application of acrylic cement, particularly in the femoral shaft, is known to cause a slight decrease in blood pressure and occasionally severe hypotension. It seems therefore advisable to observe a number of rules. The surgeon should ensure optimal mixing as long as possible outside the organism and provide adequate ventilation of the femoral shaft; the endoprosthesis should moreover be eased into its proper position. Points of importance to the anaesthetist are to ensure adequate filling of the circulating volume before introduction of acrylic cement, and prevention of even slight hypoxia. The possible contribution of the cytotoxic monomer to local tissue necrosis remains unknown.

1.2.7. Allergy

So far there have been no indications of significant allergic complications caused by acrylic cement (and particularly by the monomer methylmethacrylate) in orthopaedic applications. Sensitization to the monomer as reported in dermatological and dental literature (Slooff 1970; Charnley 1970), however, has been described in orthopaedic surgeons with contact

dermatitis (Pegum and Medhurst 1971; Blair Fries et al. 1974) and in patients after total hip replacement with the aid of bone cement (Blair Fries et al. 1974). The monomer seems to penetrate surgical gloves readily.

Until the contrary is proved, the possibility that allergic reactions with serious consequences for the patient can be provoked by the cement components or additives must be taken into account (Bloch and Hastings 1972; Müller 1974b).

1.2.8. Carcinogenic effect

Partly in view of reports on sarcomatous degeneration after application of PMMA in test animals (Contzen et al. 1967; Slooff 1970; Charnley 1970), the Food and Drugs Administration has long delayed acceptance of acrylic cement in the USA. Finally, in 1971, only one product (Simplex-P) was accepted. There has been one case report on malignant degeneration following surgical application of PMMA.

Some 18 years after application of Lucite for extrapleural plombage of a tuberculous pulmonary process, an extraskeletal chondrosarcoma was found in the supraclavicular fossa, which proved to be directly related to the fibrous capsule enveloping the Lucite (Thompson and Entin 1969).

So far as could be established, no malignant degeneration has ever been ascribed to any orthopaedic application of PMMA. However, after exposure of the human organism to recognized carcinogens it may be 20-35 years before a malignancy becomes manifest (Ott 1970; Lavorgna et al. 1972). In view of the long latent period, no definite conclusion can as yet be reached about the carcinogenic effect of acrylic cement on the human organism. Discriminate use and prolonged follow-ups, particularly on younger patients, are therefore imperative.

1.3. *Histopathological reactions*

1.3.1. Introduction

In descriptions given in the past of the effect of PMMA on living tissue, distinction must be made between tissue changes occurring in reaction to:

a. polymerized PMMA;
b. PMMA setting in situ.

All authors quoted by Contzen et al. (1967), Slooff (1970) and Charnley (1970) regard polymerized acrylic cement as a biocompatible material. The principal reaction mentioned is the development of a connective tissue membrane at the interface between PMMA and living tissue.

When this material is exposed to stress, signs of chronic inflammation are seen in the connective tissue layer, with reactive necrosis and increased remodelling in adjacent bone tissue. These reactions can increase due to disintegration of the PMMA and, together with mechanical imperfections, this ultimately led to rejection of the Judet type of Plexiglas prosthesis as mechanically and biologically unsuitable.

The situation of PMMA setting in situ – the acrylic bone cement – is a different one. It is in fact highly dubious whether this could be described as a biocompatible substance. The possible untoward local side effects of the bone cement, most important among which is the high polymerization temperature and the cytotoxicity of the non-polymerizing monomer, become manifest in contact with the living organism. The histopathological reactions which acrylic cement provokes in living human tissue can be divided into reactions in soft tissues and those in bone.

1.3.2. Reactions in the soft tissues

After total hip arthroplasty, the movable parts of the artificial joints come to be surrounded by a new connective tissue capsule which replaces the capsule which is (usually) excised during the operation. The histopathology of this new capsule, removed in revision operations or postmortems, has been described by Cotta and Schulitz (1970), Semlitsch et al. (1971), Willert (1973a), Willert and Semlitsch (1973b, 1974a), Beneke et al. (1973), Charosky et al. (1973), Masshoff and Neuhaus-Vogel (1974), Evans et al. (1974) and Brinkmann and Heilmann (1974).

Although the structure of the new capsule shows some resemblance to that of a normal joint capsule, yet an identical tissue structure is never attained. The tissue structure shows changes and is much coarser because regenerative processes in the new connective tissue are accompanied by a tissue reaction to the presence of foreign bodies. These foreign bodies are wear products of metal and plastic prosthesis components, and bone cement particles from the implant bed.

According to Willert and Semlitsch (1973b, 1974a) acrylic cement particles are a major factor in the development of and the reactions in the new capsule. They are held to contribute more to these than the wear products of the prosthesis components. In the new capsule these foreign bodies produce a typical, partly granulomatous foreign-body reaction, which varies with the degree of wear and other factors such as infection, instability of the prosthesis and excessive amounts of acrylic cement. The chronic foreign-body reaction maintained by constant addition of newly formed wear particles leads to progressive thickening and cicatrization of the new capsule, and over a long period of time this can produce

some loss of mobility in the artificial joint (Willert and Semlitsch 1973b, 1974a).

The granulation tissue tends to become necrotic, and necrotic material can be recovered in the form of cheese-like debris from large pouches which have formed in the new capsule. The wear particles are eliminated in minute amounts via the perivascular lymph interstices (Willert 1973a; Willert and Semlitsch 1973b, 1974a; Brinkmann and Heilmann 1974). If this elimination is insufficient or if the supply is too large, then this may lead in the long run to resorption of bone required for fixation of the cement, with consequent loosening of the prosthesis components (Willert and Semlitsch 1974a).

Evans et al. (1974) maintain that hypersensitivity to metal wear particles can lead to several reactions which, via obliteration of supplying blood vessels, give rise to necrosis of the bony implant bed and ultimately cause loosening of the prosthesis.

The development of this capsule should be carefully watched because it may give rise to pain, functional impairment, loosening of the prosthesis, and even possible sarcomatous degeneration (Willert and Semlitsch 1973b, 1974a; Charosky et al. 1973). Although careful studies by the authors mentioned on page 26 have fortunately failed to demonstrate malignant degeneration following the use of acrylic cement in allo-arthroplasties, Willert and Semlitsch (1973b) and Charosky et al. (1973) nevertheless stress the potential risks entailed by chronic progressive cicatrization on the basis of a foreign-body reaction in this context.

1.3.3. Reactions in bone

Charnley (1970) described the histopathological changes of bone in contact with acrylic cement, with reference to postmortem findings obtained in 23 patients who had shown an uneventful clinical course after implantation of an artificial joint, which had remained stably fixed in the bone for many years (longest period reported: 7 years). He observed that a thin layer of bone tissue (0.5 mm thick) in direct contact with the acrylic cement had become necrotic. At the interface between bone and acrylic cement a connective tissue membrane had formed which contained giant cells but showed none of the typical signs of chronic inflammation. From this connective tissue membrane a fibrocartilaginous tissue had developed by metaplasia induced by mechanical pressure. In this tissue there were sometimes ossified areas, in contact with underlying bone tissue. Dead bone had in part been replaced by new osteons which at some sites lay against the cement. However, Charnley (1970) considered above all the fibrocartilaginous layer to be essential for the transmission of forces from cemented prosthesis to bony implant bed.

On the basis of these observations and biomechanical theories (Charnley 1965), and supported by his good long-term clinical and radiological results, Charnley (1970) described acrylic cement as a safe material which – although not yet ideal – meets all mechanical and biological requirements.

Willert and Puls (1972) studied the histological reaction of bone to acrylic cement (Palacos) in 23 cases of total hip arthroplasty or hemiarthroplasty of the hip. The material was obtained at postmortem, the operations having been performed 7 days to 5 years before this examination.

In all patients whose operation dated back more than 10 weeks, the implant had borne weight and the clinical result had been satisfactory. In alle cases fixation of cement to bone was macroscopically firm, and no mobility between cement and bone tissue was demonstrable. Intimate interlocking of the cement in the interdigitations of the cancellous trabeculae was found in the metaphysis. Along the compact bone of the diaphysis and along the acetabular floor the cement did exactly follow the bone surface, but at these sites the bone architecture happens to be less suitable for fixation as firm as that is possible in cancellous bone. In these areas the cement consequently showed a much smoother surface. Necrosis was found in a thin layer of bone (up to 3 mm) surrounding the cement, and large necrotic foci were found in the cancellous bone of the greater trochanter and in the medullary cavity of the femoral diaphysis distal to the point of the stem of the prosthesis. Willert and Puls (1972) distinguished three phases in the tissue reactions to the implanted bone cement:

a. the initial phase, lasting up to 2-3 weeks after implantation;
b. the phase of reparation, extending from 3 weeks to a year or longer;
c. the phase of stabilization, which was completed after a maximum of two years.

These three phases were best described in terms of microscopic features.

In the *initial phase,* fat cells and haemopoietic cells in the bone marrow degenerated. There was lipophagia by polynuclear phagocytes, which closely resembled foreign-body cells. The cancellous bone showed necrosis of cancellous trabeculae at sites of contact with the cement. The extent of necrosis in the diaphyseal compact bone showed marked interindividual changes, ranging from an area of 0.5-0.8 mm to one-third of the inner surface of the cortex!

The phase of reparation was characterized by organization of the bone marrow necrosis to fibrosis, in which avital bone marrow elements were still demonstrable after as long as one year. The boundary with the vital bone marrow was demarcated by a hyperaemic marginal zone. Beginning in areas where the bone marrow had been revitalized, remodelling processes were taking place in the necrotic bone. Osteogenesis took place by metaplasia of connective tissue in which woven bone was deposited,

or by deposition of new bone lamellae on old necrotic cancellous trabeculae. In the diaphyseal compact bone, mostly lamellated bone was deposited on the interior wall of the haversian canals. Bone trabeculae anchored in the cement could disappear entirely due to osteoclastic bone destruction, with formation of lacunae especially in cortical bone. The bone remodelling was not confined to the primarily necrotic areas. It was observed also in cancellous or compact bone areas not directly adjacent to the implant, with predominance of bone destruction. This produced rarefaction in the cancellous bone, and porosity of compact bone. The features of characteristic osteoclastic osteoporosis could be further enhanced by pre-existent bone atrophy, as seen in elderly patients. The phase of reparation was found to be completed about 2 years after the operation. All necrotic tissue had been replaced by vital bone marrow and bone, and the bone cement was found surrounded everywhere by a thin layer of vital bone tissue.

In the *phase of stabilization,* a collagenous connective tissue membrane of 0.1-1.5 mm thickness was found as remnant of the above described bone marrow fibrosis; this membrane contained lymphocytes, plasma cells and polynuclear foreign-body giant cells. The bone marrow otherwise had a normal structure. In the bone this phase was characterized by further remodelling, with as conspicuous feature reorientation in the course of the bone trabeculae. While initially these had been arranged perpendicular to the bone cement, they were chiefly found to run more or less parallel to the cement surface in the phase of stabilization. Total bony enclosure of the acrylic was not observed anywhere in the material examined. By far the largest part of the interface consisted of bone marrow which showed a smooth or latticed meshwork structure. This bone marrow was separated from the cement by a connective tissue membrane in which, in the phase of stabilization also, haemorrhagic areas with fibrin deposits were seen.

In the initial phase the anchoring of cement in bone was most secure, even though the interlocking bone trabeculae were usually avital. Due to remodelling and reorientation of the bone trabeculae, interlocking of these trabeculae in the cement was much less in evidence in the phase of stabilization. For the most part, the bone was not in direct contact with cement but separated from it by a thin zone of flat polynuclear cells. These cells were probably to be regarded as the equivalent of the giant cells in the cement/bone marrow interface. Wherever there was more space between bone and cement in the phase of stabilization, smooth or latticed meshwork marrow was found, separated from the cement by a connective tissue membrane.

The intensity of the changes described varied from case to case, and therefore the interval required for revitalization of necrosis and the healing process also varied. The impression was gained, moreover, that after early mobilization and weight-bearing the processes of degeneration and regeneration of bone were better balanced and led to less extensive osteoporosis.

The above described findings roughly correspond with those of Charnley, but they have been differently interpreted by Willert and Puls (1972) and Willert et al. (1974b). They maintained that the implant of prosthesis with cement owes its stability to anchoring in bone. In their view connective tissue membrane, regenerated bone marrow and soft tissues grown into the cement may mechanically function as a buffer which absorbs excessive forces impinging on the implant. They believe that the haemorrhages and exudates found in the connective tissue membrane corroborate this. In their opinion, the bone cannot be expected to interlock as intimately with

the cement during revitalization of the necrotic foci as at the time of insertion of the prosthesis. According to Willert and Puls (1972), therefore, the anchoring in bone is never afterwards so firm as at the time when the cement is setting immediately after implantation!

Despite a favourable initial clinical course following a technically perfect operation, minimal movements between bone and implant can eventually occur due to diminution of the firmness of bony anchoring, as described by Charnley (1970), Willert and Puls (1972) and Willert et al. (1974b). Should the demands made upon the implant exceed the strength of the cement/bone anchoring (and this is often unpredictable), instability of the implant inevitably results. A series of microfractures in the interface upon continued stress can lead to loosening and even fracture of the cement (Homsy 1973; Willert et al. 1974b). The tissue changes in the implant bed might very well lead to late loosening of the prosthesis. It is therefore of importance to make an attempt at answering the ever-repeated question about the possible cause of this necrosis. The three possibilities suggested are: interference with the bone vascularization at operation, the high maximum polymerization temperature of the cement, and the cytotoxicity of the monomer.

1.4. *Summary*

1. Acrylic bone cement as applied in joint-replacing operations has generally fulfilled expectations.
2. Polymerization entails the development of a high temperature and the release of non-polymerizing cytotoxic monomer. At a local level, these side effects might contribute to necrosis involving a thin layer of the bony implant bed. We do not know whether this necrosis is caused by the untoward side effects of cement setting in the living organism or whether a vascular factor is (also) responsible.
3. The general pharmacological toxicity of the monomer methylmethacrylate, in the amounts commonly used in acrylic cement, is not alarming.
4. Sensitization and carcinogenic effects of acrylic cement used for orthopaedic purposes have so far been unknown, but cannot be excluded with certainty in the long run.
5. Several factors exert a negative influence on the mechanical qualities of PMMA. Shrinkage of acrylic cement could well be an unfavourable factor of some importance.
6. Cement particles can contribute to an unmistakable extent to the reactive development of a greatly thickened new joint capsule.

CHAPTER 2

THE VASCULARIZATION OF DIAPHYSEAL CORTICAL BONE AND THE REACTIONS TO DISTURBED MEDULLARY CIRCULATION

2.1. *Introduction*

The direct relationship between vascularization and osteogenesis has been recognized for centuries, but it is only in the past 25 years that attention and studies have focused on the anatomy of the osseous circulation on a microscopic level (Trueta 1968; Brookes 1971).

On the one hand this can be explained by the development of more advanced equipment to process bone and by the fact that perfected perfusion techniques have made it possible also to visualize microscopically small vessels. On the other hand, the more aggressive attitude adopted in the past few decades by orthopaedic surgery in dealing with congenital and acquired anomalies of the musculo-skeletal system may also have given impetus to the renewed interest.

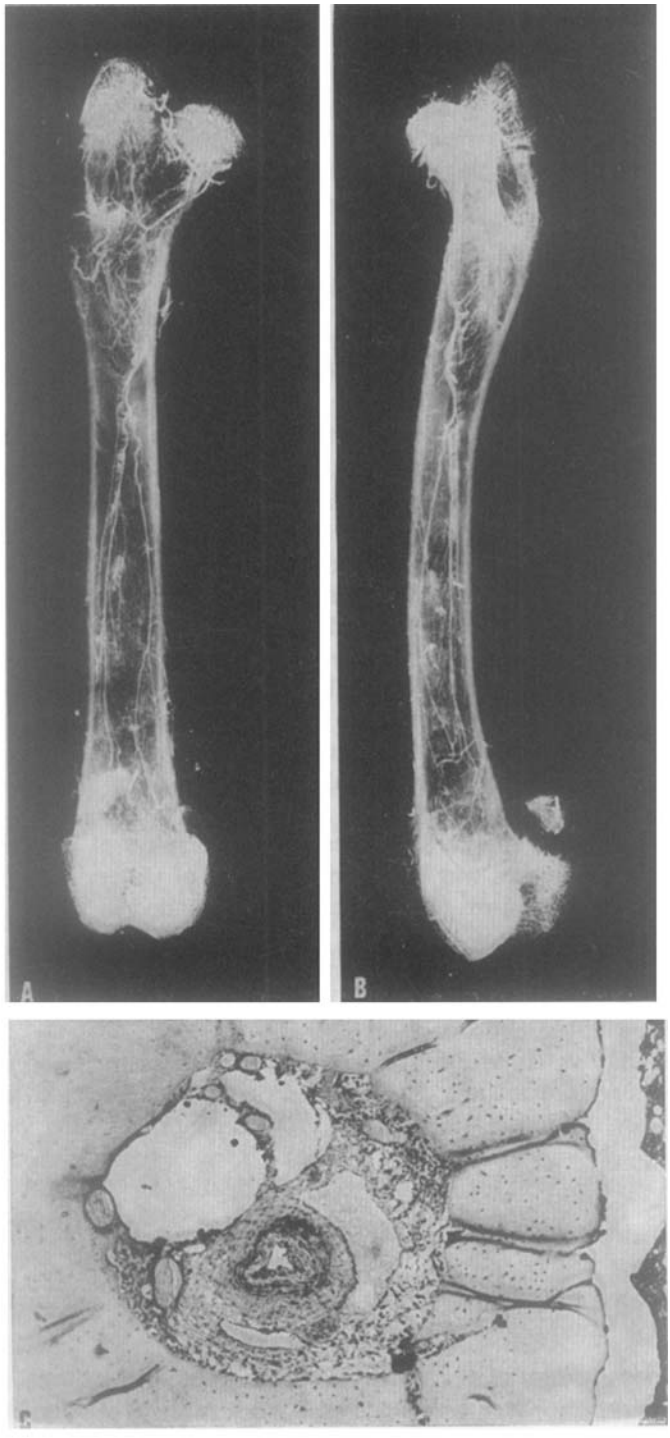
Our knowledge of the cortical circulation of diaphyseal bone in mammals, which for decades was based on the works of Langer (1876), Lexer et al. (1904) and Johnson (1927), has been expanded in the years after World War II, mainly by the results of animal experiments. It has been established that man and (test) animal, regardless of species, show the same basic patterns (Tilling 1958; Rhinelander 1962, 1972; Koekenberg 1963; Trueta 1968; Brookes 1971).

The arterial vascularization of a long bone has three sources: the nutrient artery, the metaphyseal arteries (in combination with the epiphyseal vessels after closure of the growth plate), and the periosteal arterioles.

2.2. *The vascularization of diaphyseal cortical bone*

2.2.1. Topographic anatomy of the vascularization of the diaphysis of the rabbit femur

The *nutrient artery* of the rabbit femur arises from the lateral circumflex femoral artery. Over a distance of 2 cm it extends distally along the


A
B

lateral aspect of the iliopsoas tendon (Shim et al. 1970) and enters the nutrient foramen. This foramen extends obliquely to the knee and is localized on the medioposterior side of the femoral shaft, immediately distal to the lesser trochanter (Brookes & Harrison 1957). In the foramen the nutrient artery produces no branches. Once in the medullary cavity after passing through the foramen, it divides into ascending and descending vessels whose branches supply the bone marrow and the cortex with blood (figure 2). In the cortex, arterioles which can be followed at best to a point halfway the cortex ultimately open up into the Haversian canals and Volkmann's canals, which contain one or two endothelium-lined vessels the size of a capillary (Brookes 1971; Rhinelander 1974).

Among the *metaphyseal* arteries the artery of the trochanteric fossa, which arises from the medial circumflex femoral artery, merits some attention because, according to Brookes & Harrison (1957) and Brookes (1971), it could be a second afferent vessel for the diaphysis. This artery enters through a foramen in the depth of the trochanteric fossa (Barone et al. 1973), and divides into ascending branches which supply the greater and lesser trochanter as well as the femoral neck with blood. The third trochanter (gluteal tuberosity of the femur) has no separate nutrient artery. In addition, branches of the trochanteric anastomosis and the arterial ring at the base of the femoral neck enter the metaphysis through many foramina. The distal femoral metaphysis is supplied by numerous metaphyseal arteries whose fairly thick branches penetrate the cortex through many foramina (Rogers & Gladstone 1950; Brookes & Harrison 1957; Brookes 1964).

The growing *epiphysis* has its own separate vascularization, and until completion of growth the growth plate constitutes a vascular barrier between the epiphyseal and the metaphyseal system (Trueta & Morgan 1960; Koekenberg 1963; Trueta & Cavadias 1964).

It is probably only at the capillary level that the medullary arteries anastomose with the metaphyseal vessels; after closure of the growth plate, anastomoses with the epiphyseal arteries probably do exist but have not been demonstrated with any certainty (Brookes 1971). According to Brookes (1964, 1971) this would imply that, generally speaking, under physiological conditions the diaphyseal, metaphyseal and epiphyseal systems supply only their own region with blood. According to Shim et al. (1970), the nutrient artery accounts for at least 70 per cent of the circulation of diaphysis and bone marrow, to which the other systems

←

Fig. 2: The course of the nutrient artery and the metaphyseal and epiphyseal arteries in the rabbit femur (A, B). Transverse section (C) through a normal rabbit femur at the level of the passage of the nutrient artery through the cortex. Also note the accompanying vein and arterioles and two nerves (HE x 63).

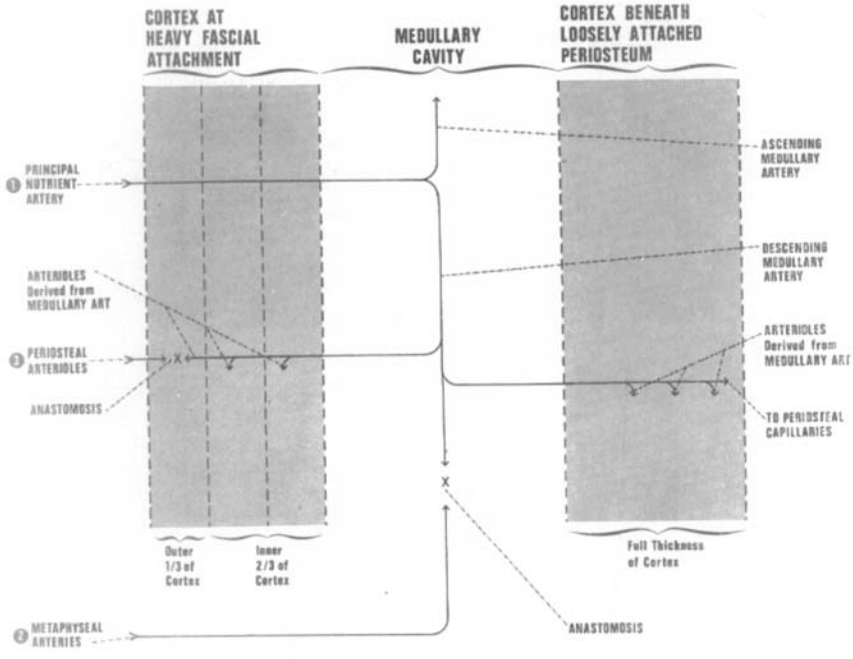


Fig. 3: Rhinelander's diagram showing the afferent vascular system of a mature long bone and its distribution.

Components 1, 2 and 3 constitute the total arterial blood supply to the diaphyseal cortex. Arrows indicate direction of blood flow.

Adopted from Rhinelander, F. W.: Circulation in Bone. In: The biochemistry and physiology of bone (G. H. Bourne, ed.), 2nd edition, vol. II, pp. 1-77. Academic Press, New York 1972 (with the publisher's consent).

contribute about 30 per cent. Under normal conditions each of the regional systems accounts for at least two-thirds of the circulation of its primary supply region (Shim 1968). Trueta (1959, 1968) also refutes the notion that these systems consist of end-arteries.

On the capillary level there is a direct communication between the *periosteal* network supplied by the periosteal arterioles, and the cortical capillaries. At sites of muscle attachment (linea aspera), where the fibrous periosteum is firmly anchored to the bone, the intramuscular and the periosteal circulation merge. The vascularization at these sites is more abundant than elsewhere in the periosteum (Brookes 1971; Rhinelander 1972, 1973).

A large number of *venous* sinusoids supplied by intracortical capillaries and medullary veins, drain into a central intramedullary canal which

extends throughout the length of the diaphysis and drains into the two venous metaphyseal systems. This central venous sinus is many times as wide as the nutrient artery but has a much thinner wall. According to de Marneffe (1951) and Tilling (1958), there are no valves in it. In the diaphysis the nutrient vein is the sole ramification (Brookes & Harrison 1957; Morgan 1959; Brookes 1971).

2.2.2. Functional anatomy

A more profound insight into the significance of the above described systems can be gained when we consider the contribution of each system to the cortical vascularization of a long bone *in the functional sense.* Two relevant theories merit a more detailed discussion.

2.2.2.1. Singular circulation concept

According to Brookes (1971) and Rhinelander (1972) the medullary circulation, made up by the nutrient artery and also partly by the metaphyseal arteries through anastomoses on a capillary level, is responsible solely for the *afferent* cortical circulation. They therefore maintain that, under physiological conditions, the direction of flow in the diaphyseal cortex is centrifugal.

The periosteal network plays a subordinate role in the afferent circulation. Possibly it contributes to the vascularization of a few superficial lamellae, and this is probably the case in immature cortical bone lined with a richly vascularized periosteum (Rhinelander 1974). Rhinelander (1971, 1973) attaches special significance to the periosteal arterioles in the vicinity of muscle attachments (linea aspera), which in this limited sector are believed to vascularize one-quarter to one-third of the outer layers of compact bone. The afferent vascular system is schematically represented in figure 3.

Certainly in mature cortical bone the periosteal vessels are to be regarded as the final link in the *efferent* system. The punctate haemorrhages which occur when periosteum is stripped off vital bone, indicate that blood leaves the cortex here. Venous blood from the *intermediate system* made up by Volkmann's canals and Haversian canals, which constitutes the link between the afferent and the efferent system, ultimately drains through cortical venous canals into the extraosseous (periosteal) vascular network (Rhinelander 1972, 1974). The periosteal capillaries, which communicate with the cortical capillaries of the superficial cortical lamellae, should also be regarded as a component part of the efferent system. It is mainly the medullary sinusoids and the central

venous sinus that are responsible for the drainage of bone marrow products. These enter the medullary venous system by virtue of the permeability of the sinus endothelium, or by phagocytosis (Brookes 1971).

The above outlined concept of a centrifugal one-way afferent circulation of cortical diaphyseal bone, originally advanced by Brookes & Harrison (1957), has been corroborated by MacNab (1957, 1958), MacAuley (1958), Jackson & MacNab (1959), and Nelson et al. (1960). And it has been elaborated and substantiated in excellent micro-angiographic studies by Rhinelander and his co-workers. Suffice it here to present a summary of their principal results.

Using fine granulated barium sulphate (Micropaque) as perfusion medium, the micro-angiograms were studied microscopically and stereoscopically, and correlated to the histology of sections originating from the same tissue slices. Throughout the healing process of non-dislocated radial and ulnar fractures in mature dogs the medullary circulation, which had remained intact, continued to dominate. External callus was supplied by extraosseous circulation (Rhinelander et al. 1962). In the case of dislocated fractures, however, with interruption of the medullary circulation, an increased periosteal or extraosseous circulation was found initially to be the primary source of vascularization for the forming callus (Rhinelander et al. 1968a). A typical feature was the course of these vessels perpendicular to the cortex. Three to six weeks after fracture infliction, the medullary circulation had regenerated and resumed its prominent place in the blood supply to the callus and the devitalized fracture fragments. This process proved to be as much accelerated as reduction of the fracture had been more exact. In radial osteotomies followed by stable plate fixation, reconstruction of medullary vessels was observed after only one week (Rhinelander 1968b, 1972). The significance of the temporarily increased extraosseous vascularization gradually diminished, and ultimately even the periosteal callus was supplied from the medullary circulation. In the final stage of remodelling the normal pattern of vascularization returned, in which medullary vessels anastomosed with the periosteal vessels where they entered in the outer layers of the cortex at fascial attachments (Rhinelander 1968a, 1968b). Even after destruction of the medullary circulation by osteotomy of the tibia (Rhinelander 1965) or the ulna (Rhinelander et al. 1967) in dogs, followed by insertion of a loose-fitting intramedullary rod, the medullary circulation proved to have enormous regenerative powers. Insertion of a tight-fitting nail after reaming of the medullary cavity through a femoral osteotomy, resulted in temporary devascularization of the entire cortex, with the exception of a small posterior sector near the linea aspera (Rhinelander 1973). The medullary circulation regenerated gradually, and finally supplied the entire cortex with blood. In the intermediate phase (8 weeks) of healing

extraosseous arterioles penetrated the periosteal callus and vascularized osteoclastic removal of necrotic bone of the anterior and both lateral cortices. This extraosseous circulation, however, was transient and was reduced to normal proportions as soon as the normal medullary system had sufficiently regenerated. When physiological conditions are interfered with, therefore, e.g. by interventions which completely block the medullary blood flow, the direction of flow through cortex may be reversed by supply from the extraosseous system. This new extraosseous circulation originates from soft tissues surrounding the fractured long bone and is a transient phenomenon (Rhinelander 1972). Göthman (1960a, 1960b) as well has shown that the extraosseous supply comes from the soft tissues. It disappears after regeneration of the medullary circulation.

2.2.2.2. Dual circulation concept

The concept outlined above has been refuted by Trueta & Cavadias (1955, 1964), Trueta (1963, 1968) and Crock (1967), one of Trueta's co-workers. They agree that the medullary system, to which the nutrient artery contributes some 70 per cent while the metaphyseal and epiphyseal arteries account for the remaining 30 per cent, is the most important system for the vascularization of the diaphyseal cortex. But they restrict this to the inner two-thirds of the compact bone, maintaining that here the centrifugal flow encounters a centripetal flow originating from the periosteal vessels, which in their opinion supply the outer one-third of the cortex. They hold that the efferent venous flow from the cortex is mainly through the medullary venous system. This view was supported by the experiments of Langer (1876), Johnson (1927), Foster et al. (1951), Göthman (1960a), Larson et al. (1961) and Koekenberg (1963).

They advanced their hypothesis on the basis of experiments with young and mature rabbit radii, in which the contribution made by each of the three sources of vascularization was determined by blocking the other two (Trueta & Cavadias 1964). For example, after blocking the metaphyseal vessels and severing the nutrient artery in young and mature animals, they found that the outer one-half to one-third of the cortex remained vital. Johnson's findings (1927) were identical. After severance of the nutrient artery and suppression of the periosteal blood flow by stripping off the periosteum and placing a thin polythene sheath around the bone, one-half to two-thirds of the inner cortex retained its vitality as long as the medullary circulation was taken over by anastomoses with the metaphyseal system. If this did not happen (young animals), then total necrosis of the cortex resulted. Similar results were reported after ligation of the nutrient artery and stripping of the periosteum in dog tibiae by Johnson (1927), in rabbits femurs by Foster et al. (1951) and

in dog femurs by Larson et al. (1961). The differences observed between young and mature animals would have to be explained on the basis of the insufficiency of the metaphyseo-medullary anastomoses in the young animals and the presence of the vascular barrier which the growth plate erects between epiphyseal and metaphyseal vessels (Trueta & Cavadias 1964).

Finally, if only the nutrient artery was left intact, most of the cortex was found to retain its vitality, and only the outer one-quarter showed necrosis. This observation, too, confirmed Johnson's experiment (1927). Trueta found evidence substantiating the existence of a dual circulation in his observation (1963, 1968, p. 167) that two or even three vessels extended in opposite direction in a single Haversian canal. This finding, however, has never been corroborated by other investigators (Brookes 1971) except Brånemark (1959). The most prominent critics of the dual circulation concept have been Brookes and Rhinelander.

2.2.2.3. Refutation of the dual circulation concept

According to Brookes (1971) the observation of cortical necrosis following stripping of the periosteum and its replacement with a plastic sheath is understandable as a consequence of venous obstruction. Rhinelander (1972) also criticized this way of suppressing physiological functions. The remarkable fact is that the opponents make use of the same weapon; i.e. both Brookes (1971, p. 122) and Trueta (1968, p. 168) find support for their arguments in a study by Nelson et al. (1960). Referring to a vascular study of human tibiae, these authors stated: „we agree with several recent studies (Brookes 1958; Brookes & Harrison 1957; MacNab 1957) that indicate that the periosteal vessels are of little significance in supplying arterial blood to the normal tibia".

More recent studies also seem to decide the issue in favour of Brookes and Rhinelander. The dominant importance of the medullary circulation was likewise convincingly demonstrated by Danckwardt-Lilliestrom (1969), Danckwardt-Lilliestrom et al. (1970a) and Olerud & Danckwardt-Lilliestrom (1971). Indian ink micro-angiography and fluorochrome labelling were among the methods used in all the animal experiments next to be described.

In the first study (Danckwardt-Lilliestrom 1969) the medullary circulation of growing and mature rabbits was destroyed by reaming, brushing or suction. After these procedures the periosteal blood vessels initially proved capable of taking over nearly the entire cortical circulation. As early as one week after the operation newly formed blood vessels had penetrated the medullary cavity. Four weeks after the operation the reaction in the periosteal vessels had diminished, and the medullary

circulation had sufficiently regenerated to revascularize the inside of the cortex. After eight weeks there was no longer any difference in luminal width and depth of vascularization of the periosteal vessels between the test side and the control side. In other words: the pattern of vascularization had returned to normal. In all animals the medullary cavity contained newly formed vessels which, in all growing rabbits and some 50 per cent of the mature animals, were identifiable as thick-walled arteries, and in all animals as normal sinusoids. After reaming and brushing of the medullary cavity in mature dog femurs, the totally destroyed vascularization was found to have started regeneration within one week. Three weeks ofter the operation the revascularization of the medullary cavity was nearly complete, and one week later it was complete. The reactive periosteal vascularization was found to have returned to normal after six weeks.

In another experiment (Danckwardt-Lilliestrom et al. 1970a), the medullary cavity of mature rabbit tibiae was reamed according to Küntscher, whereupon a dome-shaped transverse osteotomy was fixed under compression with a specially designed medullary nail. To promote rigid fixation of the osteotomy surfaces, the mid-portion of the nail consisted of a massive metal cylinder with a length of 3.5 cm and a diameter which was 100 micra smaller than that of the burr last used. However, since the medullary cavity is not circular in cross section but triangular (proximal segment) or rectangular (distal segment), it was not quite occluded by the nail used. This procedure resulted in total destruction of the intramedullary circulation. At the level of the osteotomy, the periosteal vessels too showed discontinuity over a distance of 1 cm on either side of the cut surface.

The material was divided into two groups. In the first group, bone marrow was drained by suction through a separate aperture during reaming, whereupon an osteotomy was performed and the nail inserted. In the second group there was no suction of bone marrow during reaming. Marked differences in general reactions, severity of cortical necrosis, revascularization and fracture healing were observed between the two groups. An explanation of these differences will be offered on page 45.

The vascular reaction did not differ in principle from that in the first experiment. The first group (suction) showed quick restoration of the endosteal circulation: blood vessels were observed in the medullary cavity after one week, and the medullary circulation had largely regenerated after four weeks. This process took a considerably slower course in the second (non-suction) group.

In a third experiment (Olerud & Danckwardt-Lilliestrom 1971), dog tibiae were submitted to a double osteotomy with a Gigli saw, removing an annular diaphyseal segment, whereupon the cut surfaces were filed smooth. Next, the avascular fragment was re-inserted and fixed in situ by means of a plate mounted under compression. A study of the healing

process revealed that four-fifths of the revascularization of this fragment was accounted for by the destroyed medullary vessels, which had regenerated after four weeks (shortest observation period). The presence of the plate on the medial aspect of the tibia exerted no influence on this process, because the pattern of revascularization on the lateral aspect of the tibia was identical.

2.2.3. Conclusion

The evidence supplied by the above-mentioned authors through their highly original experiments lends further substance to the assumption that the concept advanced by Brookes (1971), with the modification introduced by Rhinelander (1972) for the vascularization of diaphyseal bone, is the more plausible of the two concepts discussed. Under normal conditions the periosteal system is of subordinate importance for the cortical blood supply. Under changed conditions, however, the extraosseous vessels prove to be of transitory importance. After shaft fractures the medullary circulation remains dominant. After complete interruption of the medullary circulation the centrifugal direction of flow may be reversed temporarily and the extraosseous system of healing bone may become the principal source of revascularization of the cortex.

2.3. *Description of the reactions to disturbed medullary circulation*

The circulation in the medullary arterial system, which has been described above as the principal source of the vascularization of the diaphyseal cortex, can be partly or totally destroyed in various ways. The ischaemia caused by intramedullary procedures has a number of typical consequences for the bone. The reactions provoked in medullary cavity, cortex and periosteum are so characteristic and reproducible as to justify a joint discussion of all relevant animal experiments carried out in the past. The intensity of these reactions largely depends on the extent of the lesion, the animal species involved, and the degree of maturity of the animals. For a comprehensive review of the literature on intramedullary procedures we may refer to Danckwardt-Lilliestrom (1969, pp. 17-22) and Danckwardt-Lilliestrom et al. (1970a, pp. 3-12).

2.3.1. Medullary reactions

Medullary devascularization leads to necrosis of the bone marrow. After an intramedullary procedure, one initially finds a blood clot with,

sometimes, a few avital remnants of bone marrow. This blood clot is organized by in-growth of markedly dilatated vessels, and new bone marrow cells can be found from the end of the first postoperative week on (e.g. Danckwardt-Lilliestrom 1969). Initially there is predominantly degradation of necrotic material with, as striking features, the presence of macrophages (Foster et al. 1951; Trueta & Cavadias 1955; Richany et al. 1965) and later of polynuclear giant cells (Bragdon et al. 1949; de Marneffe 1951; Trueta & Cavadias 1955, 1964; Brookes 1960). Trueta & Cavadias (1964) found these giant cells in young and mature rabbits as long as eight and six months, respectively, after blocking the medullary circulation (cf. page 37).

Dependent on the operative lesion, a fibrous network forms in the medullary cavity (Brookes 1960; Richany et al. 1965); this contains osteogenetic granulation tissue from which woven trabecular bone can be formed (Foster et al. 1951; Brånemark et al. 1964; Richany et al. 1965; Danckwardt-Lilliestrom 1969; Lindwer 1972). As bone marrow regeneration continues, this new bone is resorbed (Danckwardt-Lilliestrom 1969).

Foster et al. (1951) and Richany et al. (1965) reported that, in their experiments, bone trabeculae were still found centrally in the medullary cavity six months after operation. None of the investigators has described cartilage formation. Only Richany et al. (1965) and Mital and Cohen (1966) have ventured to explain the appearance of new bone in the medullary cavity following intramedullary operations. They suggested that pluripotent reticulum cells proliferate and then differentiate to mature bone marrow elements. These include osteoblasts, which form trabecular bone during reorganization of the bone marrow. This bone formation in the medullary cavity was not observed in experiments in which, after extramedullary or intramedullary interruption of the medullary circulation, the medullary cavity was filled with foreign matter in the form of an intramedullary nail (Küntscher 1940, 1962; Trueta & Cavadias 1955; Göthman 1960b; Koekenberg 1963; Gustilo et al. 1964; Anderson 1965; Rhinelander 1967, 1973; Danckwardt-Lilliestrom et al. 1970a), wax (McNab 1958), plaster of Paris (Slooff 1972) or acrylic cement (Wiltse et al. 1957; Slooff 1970, 1971, 1972; Lindwer 1972, 1975).

2.3.2. Cortical reactions

Within 24 hours of an intramedullary lesion or extraosseous blocking of the medullary circulation, incipient necrosis of the endosteal cortex can be observed in the form of shrinkage and hyperchromasia of cell nuclei (Foster et al. 1951; Trueta & Cavadias 1964; Richany et al. 1965). After a week the endosteal lining has become frayed or has

disappeared, and one-third to two-thirds of the inner cortex has become necrotic (Trueta & Cavadias 1955, 1964; Richany et al. 1965; Danckwardt-Lilliestrom 1969). In these areas the bone lacunae are empty. After a while the Haversian canals and the bone lacunae dilatate, and after two weeks the partial cortical necrosis attains its maximum (Trueta & Cavadias 1964; Richany et al. 1965; Danckwardt-Lilliestrom 1969).

As described in the first section of this chapter, extraosseous vessels react by a very marked proliferation and increase in luminal width, and such investigators as Danckwardt-Lilliestrom (1969) have demonstrated this within 24 hours. Soon after, new blood vessels are observed, which penetrate the cortex from the periphery and eventually also from the medullary cavity. With these new vessels, many „bone-forming cells" appear (Brookes 1971). Although one might expect predominance of degradation of necrotic tissue in the early stages, yet few osteoclasts are observed (e.g. Brookes 1960b).

Large resorption lacunae appear, first in the middle one-third of the cortex and then gradually also further down, where the necrosis is deepest. In the inner cortex these lacunae come to occupy so much space that fragments of dead endosteal bone can be rejected as sequestra into the medullary cavity (Trueta & Cavadias 1955, 1964; Danckwardt-Lilliestrom et al. 1970a; Slooff 1970; Lindwer 1972). Large vascular spaces, sometimes filled with bone marrow, are left behind in the inner part of the cortex (Brookes 1960; Richany et al. 1965; Slooff 1970; Rhinelander 1973). These spaces produce the radiological features of osteoporosis (Brookes 1960; Rhinelander 1973). Brookes (1960) refers to this process as „medullization", and Slooff (1970) speaks of „spongiozation" of the cortex.

It should once again be clearly emphasized that the development of all these changes largely depends on the severity of ischaemia. New endosteum begins to form after about six weeks (Richany et al. 1965; Slooff 1970; Rhinelander 1973), and from this a new endosteal layer of lamellar bone is deposited. At about the same time the process of resorption of dead cortical bone also attains its maximum intensity (Richany et al. 1965; Danckwardt-Lilliestrom 1969). According to Danckwardt-Lilliestrom (1969, 1970), bone remodelling takes place by infiltration of large broom-shaped bundles of blood vessels, which anastomose with pre-existent intracortical vessels, and of vessels preceded by osteoclasts: so-called cutter-heads (Schenk & Willenegger 1963). Even though the majority of investigators report complete revascularization after about eight weeks, necrotic areas can be encountered even after many months (Foster et al. 1951; Trueta & Cavadias 1955; Gustilo et al. 1964; Richany et al. 1965; Slooff 1970) to years (Lindwer 1972). This applies in particular to older animals, in which the stimulus to recovery appears to become exhausted (Trueta 1968).

2.3.3. The periosteal reaction

The periosteal reaction of diaphyseal bone to intramedullary procedures is likewise highly typical. As described, the primary reaction is an increase in the number of extraosseous vessels. These are of greater-than-normal luminal width, take a meandering course and extend perpendicular to the cortex. After about two weeks this vascular proliferation is most pronounced (Danckwardt-Lilliestrom 1969); it diminishes after four weeks and is reduced to normal proportions after eight weeks if healing of the fracture or osteotomy can take place (Danckwardt-Lilliestrom 1969; Rhinelander 1973).

The osteogenic periosteal layer immediately shows marked cellular activity and reacts by the formation of new subperiosteal young bone. This layer of new bone may cover the entire surface of the diaphysis. Danckwardt-Lilliestrom (1969) distinguished two morphological types: a thinner layer of lamellar bone and a thicker layer of woven trabecular bone, with its trabeculae perpendicular to the cortex. Cartilage is found only at sites directly adjacent to an osteotomy or shaft fracture (i.e. never beneath intact periosteum). This formation of new bone usually attains its maximum after two to three weeks (Trueta & Cavadias 1955; Richany et al. 1965; Danckwardt-Lilliestrom 1969). In the course of subsequent weeks this young trabecular bone is gradually replaced by lamellar bone, and the demarcation between the old cortex and the new subperiosteal layer is effaced. At the same time, normalization of the extraosseous vascular reaction is observed.

Radiologically, the new bone initially has a cloudy appearance but eventually the structure becomes denser, and the entire cortex proves to have increased in diameter (Trueta & Cavadias 1954; Küntscher 1962; Slooff 1970; Lindwer 1972; Rhinelander 1973). An exception is the experimental work of Göthman (1960b) who, after insertion of an intramedullary nail in mature rabbit tibiae, observed periosteal hyperplasia in only a few instances and subperiosteal bone apposition in none, in spite of marked extraosseous vascular proliferation. Periosteal new bone formation has been given a variety of names. Such designations as subperiosteal reaction (Richany et al. 1965; Danckwardt-Lilliestrom 1969), periosteal apposition (Lindwer 1972), provisional callus (Küntscher 1940), external callus (Rhinelander 1973), new cortex (Anderson 1965; Danckwardt-Lilliestrom et al. 1970a) and secondary cortex (Slooff 1970) all refer to the same phenomenon.

The diameter of the new bone layer can be quite considerable. This, too, depends on the severity of the lesion and on the age and species of the test animal used. An interesting fact in this context is the similarity reported by Richany et al. (1965) and Kelly (1968a, 1968b) to the periosteal bone apposition seen in the long bones in pulmonary hyper-

trophic osteoarthropathy. Although vascular changes are probably the substrate of this symptom, the exact aetiological mechanism has so far remained obscure (Lipman & Massie 1964).

Several explanations have been suggested for the newly formed subperiosteal bone after intramedullary interventions.

1. It has been ascribed to chemical or physical irritation caused by the material of the intramedullary nail (Küntscher 1940, 1962) or to pressure exerted by the nail on the bone; or it has been regarded as a reaction to insufficient fixation of the bone fragments by the nail (L. & J. Böhler 1949).
2. Such investigators as Vanderhoeft et al. (1963) reported periosteal bone apposition distal to an artificial arteriovenous fistula in growing dogs. They were unable to offer an explanation.
3. Richany et al. (1965) contemplated the possibility of stasis and oedema, leading to local anoxia and prompting the formation of new bone.
4. Zucman et al. (1968) found bone marrow subperiosteally after reaming of rabbit tibiae and believed that this marrow, pressed through the cortical canals, could promote callus formation in the manner of an autograft.
5. Kelly (1968b) produced periosteal tibial bone formation by placing a tourniquet around a thigh in growing dogs. He observed increased pressure in the lateral saphenous vein and accelerated flow (clearance of ^{85}Sr) in the tibia. He regarded increased pressure in veins and capillaries as the cause of the formation of a new layer of subperiosteal bone.
6. Trueta & Cavadias (1955, 1964), who pointed out the prominent rule of the medullary circulation in the blood supply to the cortex, sought the primary cause in the ischaemia caused by intramedullary procedures, which provokes proliferation of periosteal vessels with accompanying new bone formation. Koekenberg (1963) also believed that the cause was to be found in interruption of the circulation. Brookes (1971, p. 242) likewise believed the ischaemia to be the causative factor, and he made special mention of the changed intravascular pressure relations. Local periosteal hyperaemia could be followed by an increased cortical flow rate and changes in local oxygen and carbon dioxide pressures.

As Sim & Kelly (1970) pointed out, the oxygen demand at a local metabolic level could be an important factor in blood flow regulation.

Although considerable research has been devoted to the physiology of the blood circulation in bone, and strong indications have been found that it is regulated via neural, hormonal and particularly metabolic routes, the exact mechanisms which control blood flow are still unknown (Shim 1968; Kelly 1968a; Sim & Kelly 1970).

2.3.4. Bone marrow embolism

Danckwardt-Lilliestrom (1969) maintained that the ischaemia resulting from vascular interruption is alone insufficient to explain the variations in the extent of subperiosteal bone apposition and cortical necrosis which can be observed even in the same section. There should be another factor which could cause avascularity. He was the first to demonstrate that intracortical bone marrow embolisms are an important cause of the above described changes, and also that they can explain the occurrence of variations within the same bone after a medullary lesion. As part of his experiment (cf. page 38) he compared the percentage of avascular cortex with the amount of fat embolism demonstrated in intracortical vessels with the aid of Sudan 3 staining. He found an unmistakable correlation. He assumed that, due to an increase in intramedullary pressure resulting from the intervention, bone marrow was pressed into the Haversian canals and caused their obliteration. The resulting intracortical block, he reasoned, contributed to the extent and intensity of the cortical necrosis and the reactive subperiosteal bone formation.

Subperiosteally deposited bone marrow was also demonstrated. When the bone marrow was removed by suction through a separate aperture in the distal metaphysis, thus at the same time reducing the intramedullary pressure, the vascular damage to the cortex was substantially less, and revascularization of the entire cortex was demonstrable four weeks after the operation (Danckwardt-Lilliestrom et al. 1970b).

The experience gained was confirmed in the second experiment (cf. page 39). Both cortical necrosis and subperiosteal bone formation were substantially reduced after reducing the intramedullary pressure and removal of the bone marrow by suction. It was also found that consolidation of the osteotomy was considerably quicker when the intracortical circulation was not blocked by fat embolism.

The occurrence of fat embolism following intramedullary procedures is not confined to the osteotomized bone. Olerud et al. (1969) found massive fat emboli in the femoral vein after reaming a rabbit tibia. In the second experiment of Danckwardt-Lilliestrom et al. (1970a) described on page 39, the mortality due to pulmonary fat emboli was 11 per cent in the non-suction group; and 10 per cent of the animals showed unmistakable signs of systemic fat emboli, but survived.

The possible correlation between high intramedullary pressure and pulmonary fat embolism has also forcefully come to the fore in a different context. Postmortems on patients who had died of acute cardiac arrest during a total hip or knee arthroplasty or hemiarthroplasty of the hip in which bone cement was used, revealed massive fat and bone marrow embolism in the lungs (Milne 1973; Dandy 1974; Herndon et al. 1974).

This was initially interpreted as a possible side effect of the acrylic cement used, and special importance was attached in this respect to the lipophilic monomer (page 21).

Recent animal experiments carried out by Kallos et al. (1974b) have confirmed, however, that the occurence of pulmonary bone marrow emboli is a direct result of an increase in intramedullary pressure. No effort was made to establish the extent to which acrylic cement constituents, too, may influence the occurrence of fat embolism (cf. page 62).

2.4. *Summary*

Under physiological conditions the arterial afferent vascularization of the diaphyseal cortex of a long bone is dependent on the medullary circulation. This is supplied by the nutrient artery (arteries), and in part by anastomoses with the metaphyseal arterial system. The flow is centrifugal. The periosteal vessels are thought to be of subordinate importance in this vascularization, only a small outer cortical sector near the sites of muscle attachment (linea aspera of femur) being supplied by periosteal vessels.

Intramedullary procedures interfere with this pattern. Depending on the severity of the lesion involved, the endosteal circulation is partly or totally interrupted, thus depriving the cortical bone of its principal source of blood supply. Moreover, the cortical ischaemia is also in part determined by the occurrence of intracortical bone marrow emboli. These are as much more numerous as the intramedullary pressure during the intervention is higher (Danckwardt-Lilliestrom and co-workers). The reaction pattern of, in succession, the diaphyseal cortical circulation, the periosteum, the cortex and the medullary cavity is characteristic and reproducible. It is largely dependent, however, on the severity of the lesion, the increase in intramedullary pressure involved, the animal species used and the age of the animal.

To some extent the extraosseous circulation of healing bone is capable of taking over the circulation in the diaphyseal cortex. The afferent flow becomes temporarily centripetal. Proliferation of the extraosseous vessels is accompanied by the formation of a subperiosteal layer of young bone over the entire cortical surface. The inner cortical layers become necrotic

and, depending on the severity of the ischaemia produced, this necrosis can involve almost the entire cortex. The avascular bone marrow also becomes necrotic.

During the repair phase the medullary circulation proves to have enormous regenerative powers, and after a while it can entirely resume its function in the cortical blood supply. This concludes the importance of the temporary extraosseous vessels as a collateral system, and the rapid formation of young bone likewise subsides. This subperiosteal bone layer matures and is partially incorporated in the old cortex. In the cortex, dead bone is resorbed through invasion of new vessels, and replaced by new bone; for instance, a newly formed endosteum deposits a layer of lamellar bone. This process can take a very long time, and is associated with the formation of large vascular spaces which may contain bone marrow, and with sequestration of necrotic endosteal bone to the medullary cavity. During the reorganization of the bone marrow after reaming, macrophages and giant cells may be strikingly numerous. Also, scattered spicules of bone may form centrally in the medullary cavity, to be eventually resorbed.

Finally, it has been found that an intramedullary procedure can lead to systemic fat or bone marrow embolism as a result of a local increase in pressure.

CHAPTER 3

DESCRIPTION OF THE EXPERIMENT

3.1. *Objective, argumentation and design*

The objective of the investigation to be described was to establish, on the basis of animal experiments, which local side effects of acrylic cement cause the histological changes in cortical bone.

In the analysis of these side effects of acrylic cement, they had to be distinguished from the reactions which occur in cortical bone after interruption of the medullary vascularization, as described in chapter 2.

To begin with, the effect of interference with the medullary vascularization had to be studied in the model chosen for this study: the rabbit femur. Next, it had to be established which histological changes occur in cortical bone when acrylic cement is implanted in addition. For this purpose the two principal local side effects – those of the high polymerization temperature and the cytotoxicity of the monomer – had to be separately compared with the tissue reactions caused by interruption of the medullary vascularization.

For a separate study of the high temperature effect, we had to look for an acrylic cement which showed no or only a negligible rise in temperature at a constant monomer content. The effect of this cement had then to be compared with the reactions caused in bone by commercial acrylic cement. A simple „subtraction" would then give the tissue damage due to the effect of the high maximum polymerization temperature.

For a separate study of the monomer effect, an acrylic cement had to be found which contained a monomer excess but showed no rise in temperature. For this, too, we had to establish the extra effect in tissue damage besides the effect of interruption of the medullary vascularization. The reactions thus obtained had then to be compared with those produced by cement with a low maximum polymerization temperature and a normal monomer content.

A supplementary study had to be made of the additional deleterious effect of monomer leaking from set cement (so-called residual monomer: 2-5 per cent which remains in cured PMMA, page 21). The results thus obtained had to be compared with those produced solely by interruption of the medullary vascularization.

In this manner, it was argued, it would be possible to gain from the rabbit femur an impression of which reactions in diaphyseal cortical bone are caused by:

1. interruption of the medullary vascularization;
2. cytotoxicity of an overdose of monomer;
3. cytotoxicity of a very small amount of monomer which leaks from a set cement mass;
4. high polymerization temperature of acrylic cement setting in situ.

In order to separate these four effects, different cement types were used (section 3.2). The test animals used in this study (section 3.3) were divided into seven different groups (section 3.6) after the operation (section 3.4).

In order to study the changes in the vascularization of diaphyseal cortical bone, the lower limbs of the test animals were perfused with a fine granular barium sulphate suspension after sacrifice (section 3.7). Vascularization patterns were studied radiologically (section 3.8) and microangiographically (section 3.10).

The histopathological changes of cortical bone were studied after processing the femurs for histological (section 3.9), fluorescence-microscopic (section 3.11) and autoradiographic (section 3.12) examination.

3.2. *Cement types*

Three types of cement* were used in the experiment:

a. commercial acrylic cement (Palacos and Sulfix-6);
b. a catalyst-free „acrylic cement" obtained from one of the commercial plastics (Palacos and Sulfix-6);
c. a modified acrylic cement (Sulfix-6 with CMC gel).

3.2.1. Palacos and Sulfix-6

The properties of Palacos, which has been used in Europe for many years, can be presumed known. The ratio polymer powder with radiopaque contrast medium: monomer fluid is 2:1. The conventional packings contain about 40 g powder and 20 ml fluid.

* Palacos® and Sulfix® were respectively supplied by Kulzer & Co GmbH, 638 Bad Homburg v.d.H., Germany, and Sulzer Bro. Ltd, -CH-840 Winterthur, Switzerland.

Sulfix-6 is a new commercial product described by the manufacturer as different from products marketed earlier (Palacos, CMW and Simplex-P) in that it has a number of improved properties. It is somewhat more finely granular and has a longer working time (Debrunner and Wettstein 1975a); because 15 per cent of the fluid consists of the co-polymer n-butylmethacrylate, there is an indication of a (not significantly) lower maximum polymerization temperature at the cement surface (Debrunner 1974), and its lower porosity (Semlitsch 1975) is believed to give it slightly better mechanical properties than the other commercial acrylic cements. There are no differences in monomer release (Debrunner and Wettstein 1975b), tissue toxicity of the cured product (Semlitsch 1973) and volumetric changes during polymerization (Debrunner 1975). As in Palacos, zirconium dioxide has been added to the polymer powder as radiopaque contrast medium. The ratio polymer powder : monomer fluid is 5:2. Conventional packings contain 40 g powder and 16 ml fluid.

3.2.2. Catalyst-free „acrylic cement"

As described in section 1 of chapter 1, polymerization occurs by virtue of the presence of an initiator in the powder and an activator in the fluid. When the benzoyl peroxide (initiator) is washed out with methanol, no polymerization reaction occurs: the end-product becomes neither warm nor hard. One obtains a rubber-like substance which remains malleable for days but, exposed to air, sets after about a week due to evaporation of the monomer; this is accompanied by an unmistakable decrease in volume. We had the disposal of polymer powder and monomer fluid without catalyst system. Since no polymerization takes place in this „cement", an overdose of monomer remains in it (about 30 volume per cent). By eliminating the high polymerization temperature we could separately study the cytotoxic effect of this excess of monomer.

3.2.3. Modified acrylic cement: Sulfix-6 with CMC gel*

Next we looked for a cement which would enable us to eliminate the factor polymerization temperature. Initially we succeeded in reducing the maximum polymerization temperature by adding water when mixing the cement. Due to phase separation, however, no homogeneous distribution of water and cement could be obtained. Moreover, the mixture could not be used in the syringe. By using a high-viscosity gel, J. R. de Wijn Chem. E. (Department of Dental Materials, Nijmegen University)

* Sulzer Bro. Ltd, Winterthur, Switzerland - patents pending.

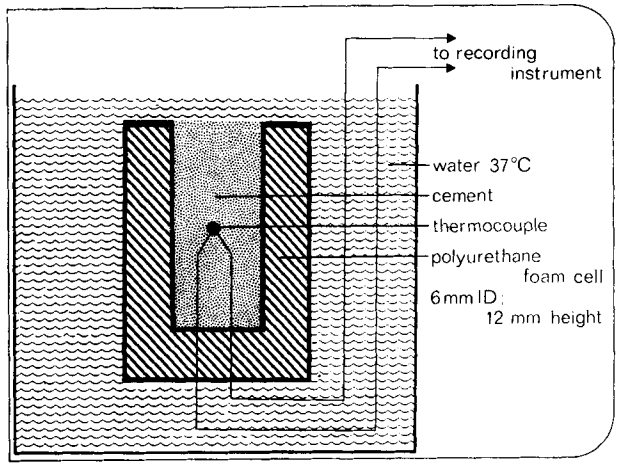


Fig. 4: Arrangement for measuring the maximum temperature in polymerizing cements.

succeeded in reducing the maximum temperature without affecting the manageability of the mixture.

To the Sulfix-6 powder and fluid in the ratio indicated by the manufacturer, an amount of high-viscosity CMC gel was added to make a mixture with 36.5 per cent (weight) gel. The polymer powder : CMC gel : monomer fluid ratio was 5:4:2. Gel phase and resin phase in this mixture remained well-distributed, which is to say that the gel formed a smooth outer layer on the surface.

In the centre of a test rod (fig. 4) of 0.5 cu cm the maximum polymerization temperature of this cement remained between 50° and 60° C. In a simular test rod the commercial cement attained a temperature of 80-100° C (fig. 5). In a globule of CMC cement kept in the hand, hardly any rise in temperature could be felt, and the temperature in vivo could be expected to be even lower because of the favourable heat release at the cement/bone interface (Debrunner 1975; Biehl et al. 1974). There were therefore sound reasons to presume that the temperature effect had been drastically reduced.

It was likewise to be expected that the percentage of nonpolymerizing monomer would equal or slightly exceed (due to the low polymerization temperature) that in the conventional commercial bone cements.

The biphasic mixture of gel and cement will develop what may be described as a gel-filled porosity, and this of course influences the mechanical properties also. Since the aqueous gel phase is biodegradable (dissolves), it could be maintained that *a porous cement* had been ob-

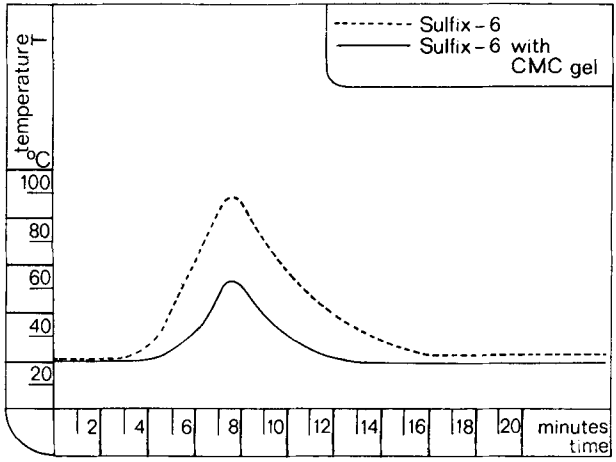


Fig. 5: General shape of temperature-time curve in laboratory tests. The polymerization time is the same for both types of cement.

tained! The pores communicated and so formed a system with so-called open porosity, in contrast with the closed porosity of commercial acrylic cements (fig. 6). Porous implant materials, with all their advantages and disadvantages, are currently in the centre of interest. For a brief discussion we refer to chapter 5. For the design of this study it was of primary importance that a manageable cement was obtained in which the high maximum polymerization temperature was eliminated while the percentage of non-polymerizing monomer remained about the same.

The gel was a solution of 5 per cent sodium carboxymethylcellulose (CMC)* in water. A 1 per cent aqueous solution of this CMC has a viscosity of 400 centipoise (CPs). The viscosity of the 5 per cent CMC gel used, as measured with a Brookfield viscosimeter at 3 rpm, was about 120.000 CPs.

Carboxymethylcellulose is extensively used in the foods industry, as wallpapering glue, in rayon yarns and as constituent of synthetic detergents. In pharmacology it is used as adjuvant in oral preparations and eye lotions, and as catheter lubricant. So far as could be established, its toxicity to vascularized tissues is negligible.

3.2.4. Sterilization

The polymer powder received from the manufacturer in the unsterilized form (Palacos and Sulfix) was divided into 10 g fractions which

* Nymcel ZHF30, Nijmegen, Netherlands.

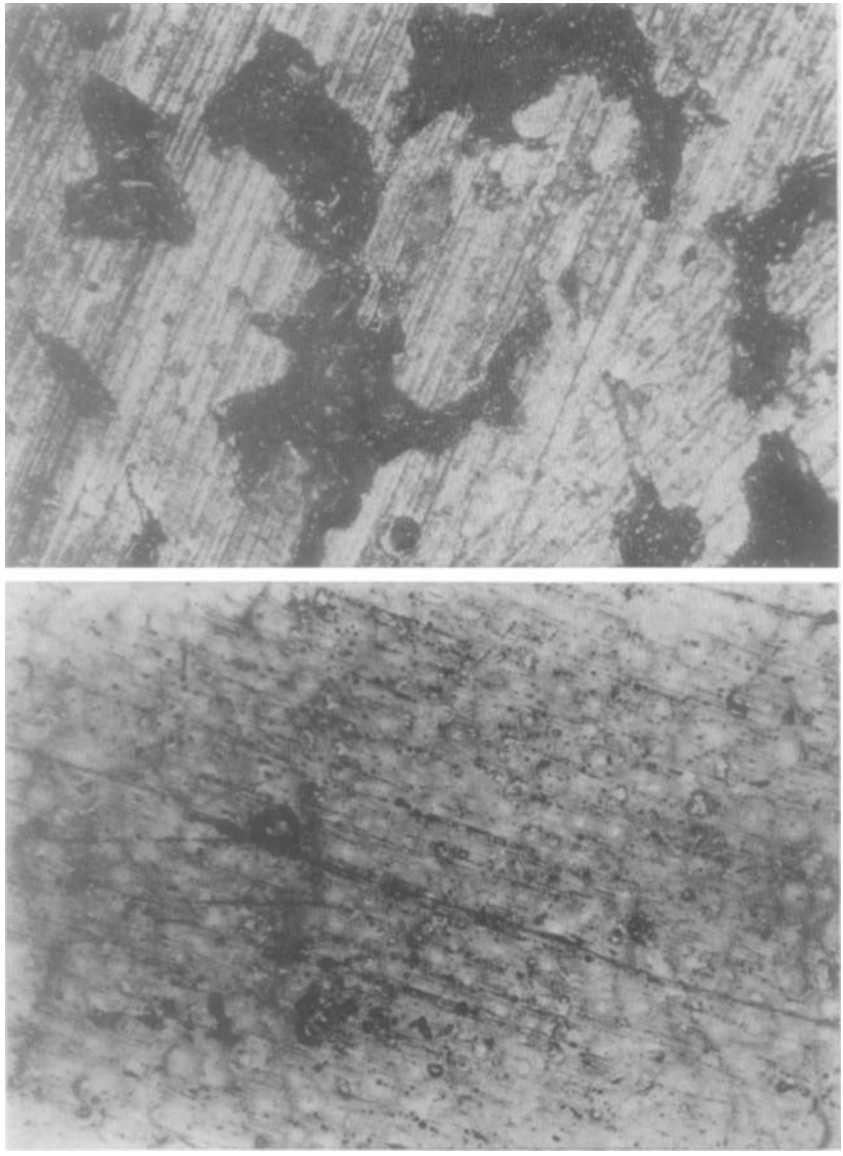


Fig. 6: Incident light microscopy (60 x). In the modified cement (above), the dark areas represent the gel phase and the lighter areas the resin phase; 35 per cent porosity. In the commercial cement (below) the dark areas represent the normal porosity as a result of air inclusion and marked evaporation of monomer during polymerization; the lighter areas represent the resin phase; 5 per cent porosity (with acknowledgement to J. R. de Wijn Chem. E.).

were double-packed and gas-sterilized (ethylene oxide). In view of the toxicity of ethylene oxide (O'Leary and Guess 1968), the sterilized powder was not permitted to be used until 2 x 24 hours after sterilization (O'Leary et al. 1968; Zagar 1972). No bacterial growth was ever found in samples which were bacteriologically examined. The monomer fluid supplied by the manufacturer was sterile, and was divided into pre-sterilized bottles under aseptic precautions. The amounts for Palacos and Sulfix-6 were 5 ml and 4 ml, respectively.

The CMC solution in water was sterilized in saturated water vapour at 100° C in a glass jar for 30 minutes and then, in a semi-liquid state, divided into sterilized eye-ointment tubes under aseptic precautions. Because the viscosity diminished during sterilization due to thermic degradation of the CMC polymer (breakdown of chain length), we used a high-viscosity CMC sodium gel to start with. This ensured that the product obtained after ultimate mixing with Sulfix-6 had the desired consistency.

3.3. *Test animals*

The test animals used in the experiment were 166 young adult rabbits of the New-Zealand White strain. They were selected because a large number of animals were needed and because adequate data are available on the vascularization of the rabbit femur. The animals were about 4 months old, in good health, averaging 2465 g (range 1900-3400 g) body weight immediately before operation. All were obtained from the same breeder and arrived at the animal laboratory at least 14 days before being used in the experiment. They were accommodated in individual cages with grid floor, received water ad libitum and were fed a standard diet (standard mixture: L.K. = 01 Hopefarms n.v., Woerden, Netherlands).

The rabbits were randomly taken from the various litters and divided over the various groups (section 3.6). The sex distribution was likewise at random.

In 4-month-old rabbits the growth plates have not yet closed. The expected total longitudinal growth of the average rabbit femur is indicated in fig. 7. The curve shown corresponds with the longitudinal growth pattern of the rabbit tibia indicated by Heikel (1960), in which the initially linear growth curve likewise declines after 100 days.

3.4. *Operative technique*

The animals were fasted during 24 hours preceding the operation. Anaesthesia was induced by intravenous injection of 0.5 mg atropine and 30 mg/kg pentobarbital sodium (Nembutal, S. A. Abbott n.v., Amster-

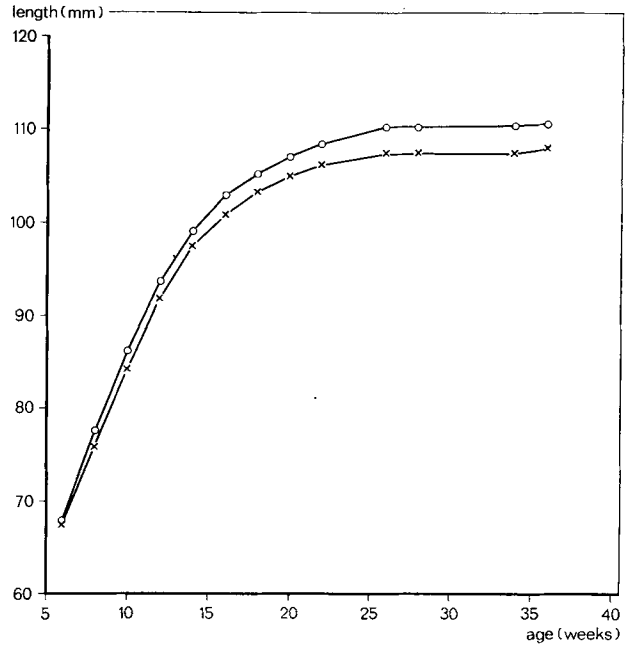


Fig. 7: Longitudinal growth curves of the rabbit femur. At age 6, 8, 10, 12, 14, 16, 18, 20, 22, 26, 28, 34 and 36 weeks, X-rays were made of both femurs in a measuring-box especially designed for this purpose, under general anaesthesia. In this box the distances between focus and plate (66.5 cm) and between femur and plate (5.7 cm) remained constant. On an OPTOCOM measuring-table the coordinates (most distal end of lateral femur and most proximal end of greater trochanter) were established, whereupon the computer calculated the distances and plotted the mean curve for both femurs of 5 male (topcurve) and 5 female (bottomcurve) New Zealand White rabbits. For calculation of the true femoral lengths, the values indicated in the two curves must be corrected by factor 0.914 (from a doctoral thesis by H. M. van de Sandt: Circumferential sectioning of the periosteum - in press).

dam). At the same time each animal received 50 mg/kg oxytetracycline (Terramycin, Pfizer Ltd, Sandwich, England) by intravenous injection as fluorochrome.

After intubation the entire left hindleg was shaved and washed with Bethadine iodine soap and 70 per cent alcohol. The animal was put on the table on its right side, lying on a water-heated mat and connected to a Keuskamp Amsterdam Infant Ventilator for ventilation with a mixture of oxygen and nitrous oxide (ratio 1 : 2) and 1 per cent halothane (Fluothane).

Sterility was ensured by observing precautions normally taken for any surgical intervention. The left thigh was painted with iodine and, after sterile

draping, an incision of 1-2 cm was made over the greater trochanter. This was stripped of its periosteum, and a manual drill with a diameter of 3.2 mm was then used for cautious reaming of the medullary cavity.* The drill was introduced over a distance of 3 cm, whereupon the correct direction was determined with a guide-wire. Drilling was then continued as far as the femoral condyles. The medullary cavity was further widened with the aid of a second manual drill with a diameter of 4.0 mm. Owing to the physiological antecurvature of the femur, reaming with this straight drill was possible only to about halfway the medullary cavity. It was needed, however, because otherwise the Teflon tubing used for suction and cementing could not be readily inserted as far as the knee. Via a second incision of 1 cm immediately proximal to the lateral femoral condyle, the periosteum was opened by means of a small longitudinal incision; the capsule was slightly pushed back and a burr hole was drilled in the cortex with a manual drill (burr diameter 1.6 mm).

A length of strong Teflon tubing (external diameter 4 mm, internal diameter 2 mm) was then inserted into the medullary cavity from the proximal end, connected with a suction tube, and slowly pushed down as far as the knee. The diameter of this tubing was such that some pressure was required to insert it, and it was felt to scrape along the wall of the medullary cavity. The tubing pushed most of the bone marrow away in front of it and this escaped through the vent near the lateral femoral condyle. Bone marrow remnants were sucked away by moving the Teflon tubing up and down five times, until only clear blood was being sucked up. After reaming and suction of the medullary cavity, the operation was continued in one of the following three ways:

a. *Closure.* Fascia and skin of both incisions were closed with linen sutures while the animal was recovering from the anaesthesia. After detubation the animal was placed on a blanket near a radiator in order to prevent heat loss.

b. *Insertion of a loose-fitting intramedullary rod* (Palacos®). A Palacos rod (diameter 3-4 mm, length 6-7 cm) was inserted from the proximal end and pushed in as far as the femoral condyles. Any part still protruding was cut off with bone forceps. The rods had been fashioned under sterile precautions a few hours earlier and had been kept sterile. They could be expected to contain 2-5 per cent residual monomer (Smith and Baines 1956; Kutzner et al. 1974a). The possible tissue-damaging effect

* The use of a powered drill was deliberately avoided because thermic damage cannot be excluded with any certainty (Danckwardt-Lilliestrom 1969; Matthews and Hirsch 1972; Rhinelander 1974).

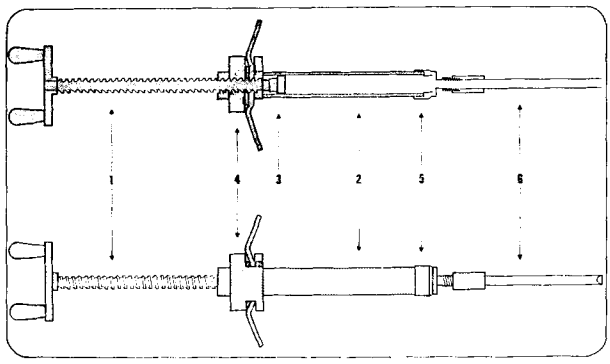


Fig. 8: Schematic representation of the modified Impregum® syringe for cementing the medullary cavity. Screw spindle (1) with piston (2). Cap (4) with bayonet joint fitting the cylinder (3). Threaded nozzle with conical end and hose connection (5). Teflon tubing (6) with an external diameter of 4 mm and an internal diameter of 2 mm.

of monomer evaporating slowly in small amounts of set acrylic cement could thus be studied. The operation was completed as indicated sub a).

c. *Cementing* of the medullary cavity. Bone cement (cf. section 3.2) was prepared and, in a dough-like state, pressed into a modified Impregum® syringe (ESPE GmbH, Seefeld, Austria). The syringe had been modified (fig. 8) to correspond with the cement syringe designed for hip surgery by Slooff (1969, 1970). Syringe and Teflon tubing could be sterilized. With the aid of a filler ring and plastic piston the cement was pressed into the cylinder until this was three-quarters full. The syringe was then mounted and the screw spindle tightened until the acrylic cement emerged from the end of the tubing. At that time the suction tube was replaced by the Teflon tubing of the cement syringe, which was inserted into the medullary cavity as far as the femoral condyles. Slowly rotating the syringe, the entire medullary cavity was filled from the distal to the proximal end, while at the same time the syringe was slowly retracted. This did not require much force because the tubing was pushed proximally by the cement filling the femur (fig. 9). Usually, a thin trickle of cement escaped from the vent-hole, and remnants of bone marrow (if any) escaped with it. The insertion and filling were done slowly, so that it took about 1 minute for the femur to be filled entirely. Dependent on the size of the animal, the amount of cement introduced was 2-3 ml. During this phase of the operation care was taken to ensure that the anaesthetic

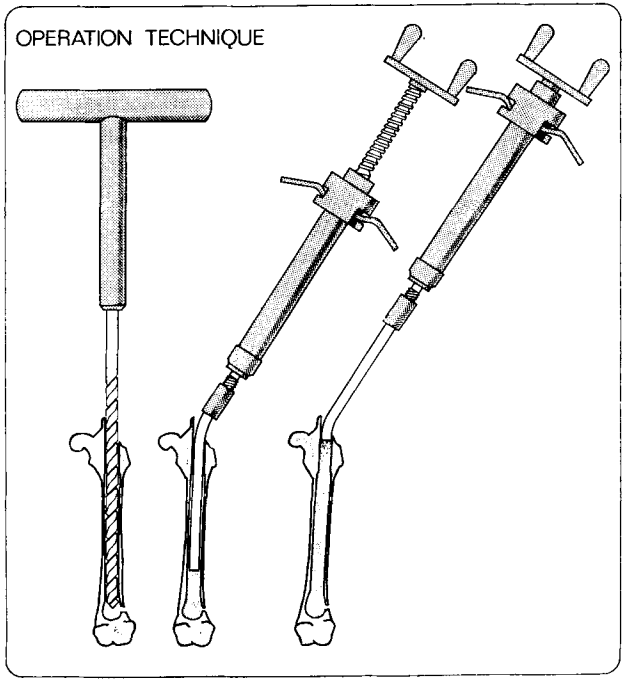


Fig. 9: Operative technique. Explanation in text pages 56, 57.

was not allowed to become too deep. The operation was completed as outlined sub a).

All operations were performed by the author, assisted by a biotechnician who gave the anaesthetic and an intern in orthopaedics. The operation was simple and readily reproducible. In all cases without exception the leg thus treated was capable of normal weight bearing after the operation; this could be checked because the animals had to stand erect on their hindlegs in order to reach their drinking-bottle. There were no infections in the area of the operation, and no wound dehisence occurred.

3.5. *Preliminary experience and explanation of the operative technique used in the ultimate experiment*

3.5.1. Introduction

In order to gain an impression of the problems which might pose themselves during the experiment, a test group of 25 rabbits was used, in

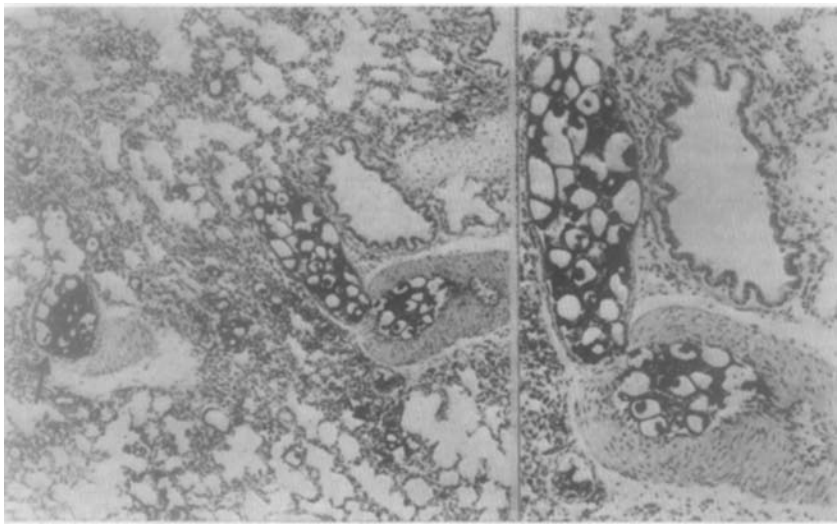


Fig. 10. Bone marrow embolism. Photomicrograph of representative lung area (x 40). The dark material is Sudan 4-stained fat and bone marrow.
The arrow points to a possible shunt between the arterial and venous system. The detail (x 70) is of an artery in parabronchial localisation with a dilated small branch. The wall of this branch is markedly thinned.

which the medullary cavity was filled with Palacos after reaming and suction. The operative technique initially used differed from that described in section 3.4. as used in the ultimate experiment: no vent-hole was drilled near the lateral femoral condyle, and the tubing of the syringe was not filled with cement to its very end.

In the first 7 animals operated on, peroperative and immediate postoperative mortality was high. One animal had to be sacrificed because of a femoral shaft fracture which had occurred during reaming of the medullary cavity. During or shortly after introduction of the cement, three animals showed pale sclerae and dilatation and downward displacement of the pupil, as well as an irregular heartbeat. Ventilation with pure oxygen and external heart massage brought no improvement. Spontaneous respiration was not resumed. There appeared to be a respiratory standstill, the irregular heartbeat continuing for a few more minutes.

During postmortem examination of the first animal to die in this manner, no macroscopical changes were seen. However when the right ventricle was opened under water, there emerged air bubbles. That air embolism had occurred was not surprising because an air column of about 7 cm length was introduced under pressure into the vascular bed, which had already been damaged as a result of the reaming. This expe-

rience taught us therefore to fill the entire length of Teflon tubing with cement before introducing the cement syringe.

Of the other two animals, one developed irreversible shock shortly after insertion of the Teflon suction tube, and the other died shortly after introduction of the cement. The deaths of these two animals (with macroscopically normal parenchymal organs at postmortem), however, could not be explained by an air embolism. In these animals, massive fat and bone marrow embolisms were found in the lungs when frozen sections were stained with Sudan 4 (fig. 10).

These complications did not occur when a lateral vent-hole was drilled in the distal segment of the femur, whereupon the cement was slowly introduced. In view of studies by Danckwardt-Lilliestrom (1969) and Danckwardt-Lilliestrom et al. (1970a, 1970b), it seems plausible that the cause of these fat embolisms had to be an increased intramedullary pressure during the operation. In order to estimate the maximum possible increase in intramedullary pressure during the operation, this pressure was measured via a simple system in the next 18 animals. It was also decided to monitor the ECG routinely.

3.5.2. Determination of intramedullary pressure with simultaneous electrocardiographic registration

3.5.2.1. Method:

A burr hole was drilled immediately proximal to the lateral femoral condyle; a screw thread was tapped and a modified hollow cortex screw (ASIF)* with a length of 20 mm was inserted and tightened as far as the opposite cortical layer. Two apertures were made at the end of this screw. Via a water-filled cannula, the screw was connected with a likewise water-filled standardized manometer. The increase in intramedullary pressure was measured during insertion of the suction tube, suction of the medullary cavity, introduction of the cement, and setting of the cement.

Throughout the operation, standard leads 1, 2 and 3 on 4 legs were used to register the heart action with the aid of a Mingograf 81 electrocardiograph (Elema-Schönander, Stockholm, Sweden). Arterial blood pressure, central venous pressure, cardiac output and blood gas values were not registered.

3.5.2.2. Results:

In 6 of the 18 animals the screw proved to occlude inadequately, and no pressure was measured. In the remaining 12 rabbits the pressure rose

* Association for the Study of Internal Fixation, Davos, Switzerland.

by a few tenths of an atmosphere during insertion of the suction tube and during suction. A marked increase in intramedullary pressure occurred while the cement was being introduced. It was found that the pressure could be regulated by adjusting the rate of introduction of the cement. The mean rise in pressure in the 12 animals was up to 1.4 atmosphere. During rapid introduction of cement the pressure could rise to 2.5 atmospheres (end of the manometer scale). The increase in pressure sometimes persisted for some considerable time once the medullary cavity was completely filled (partly because the apertures in the screw were as a rule also filled with acrylic cement).

In each of the 12 animals the ECG showed accelerated sinus rhythm and lowering of the ST complex, indicative of coronary insufficiency. No straightforward correlation was established between the level of intramedullary pressure measured and the severity of the ECG changes. The 6 animals in which the system leaked, showed no ECG changes. Three animals in which the pressure rose to 1.8, 2.3 and 2.5 atmospheres, respectively, succumbed as a result of histologically verified pulmonary fat and bone marrow embolism. In these animals the ECG features were those of a changed electrical heart axis, increasing atrioventricular block with nodal extrasystoles, repolarization disorders and intraventricular conduction disorders (broadened QRS complex).

3.5.3. Discussion

The method used to measure intramedullary pressure had some unmistakable disadvantages: a manometer is not a precision instrument; registration is not possible; the occlusion ensured by the screw in the bone was often insufficient (with as a result a pressure measured too low); and the apertures in the screw filled with bone marrow remnants and sometimes with acrylic cement. These measurements were exclusively carried out to gain an impression of the rise in intramedullary pressure, and of the extent to which it could be „regulated" by quicker or slower tightening of the screw spindle of the cement syringe.

Even though these results, which closely resemble those reported by Marsman et al. (1975), warrant no absolute or generally valid conclusions, yet, they helped in setting up the ultimate experiment. After reaming of the medullary cavity, a venthole was drilled immediately proximal to the lateral femoral condyle in all animals used in the ultimate experiment. This was done in order to:

1. prevent an excessive rise in intramedullary pressure;
2. prevent massive pulmonary embolism;
3. ensure more complete removal of medullary contents;

4. minimize the risk of intracortical bone marrow embolism which, through obliteration of vascular canals, can greatly contribute to cortical necrosis (Danckwardt-Lilliestrom et al. 1970b).

In the elegant experiment which Kallos et al. (1974) published after completion of this study, the direct correlation between high intramedullary pressure and pulmonary fat embolism was incontrovertably demonstrated.

During digital introduction of acrylic cement and a stainless steel rod in the cement mass into dog femurs they measured intramedullary pressures of 290-900 mm Hg; with the aid of a tracer (^{99m}Tc macroaggregated albumin) injected into the medullary cavity they established that medullary contents appeared in the lungs within 10-120 seconds. When a vent-hole was made in the femur, the intramedullary pressure did not rise beyond 1-93 mm Hg and no increase in radioactivity was observed in the lungs. Histological examination revealed fairly large fat-globules in the lungs of dogs with non-vented femurs, whereas only a few small globules were observed in the lungs of dogs whose femurs had been vented previously.

3.5.4. Conclusion

In view of preliminary experience gained with 25 rabbits used as a test group, we opted for drilling a vent-hole in the lateral distal femur in all animals used in the ultimate experiment.

3.6. *Division into groups*

The animals were classified according to type of operation and type of acrylic cement used (table 1). The seven groups included 18 animals each (table 2). Intercurrently deceased or disqualified animals (section 3.13) were replaced. As described in section 3.4, the operation was always performed on the left femur, the right femur serving as control. An exception was made for groups 1 and 3a, which represent bilateral

Table 1. Grouping according to type of operation.

Group	Type of operation
1	Reaming and suction of medullary cavity (RS)
2	RS + Palacos rod
3a	RS + commercial acrylic cement (Palacos)
3b	RS + commercial acrylic cement (Sulfix-6)
4a	RS + acrylic cement (Palacos) without catalyst
4b	RS + acrylic cement (Sulfix-6) without catalyst
5	RS + modified acrylic cement: Sulfix-6 with CMC gel

Table 2. Classification of numbered animals per group and per period of observation.

Period of observation	Group 1	2	3a	3b	4a	4b	5
week 1	R 6417	6538	L 6417	6439	6401	6428	6474
	R 6420[2])	6546	L 6420	6440	6402	6429	6475[2])
week 2	R 6412	6600[1])	L 6412	6432	759	6405[1])	6476[1])
	R 6413[1])	6542	L 6413	6453[1])	760	6406	6477
week 3	R 6410	6595	L 6410[3])	6451	754	6409	6492
	R 6411[3])	6545[3])	L 6411	6452	755	6414[3])	6493[3])
week 4	R 6422	6596	L 6422	6449	6403	6415	6494
	R 6423	6544	L 6423	6450	6404	6416	6495
week 5	R 6418	6597[2])	L 6418	6430	752	6426	6496
	R 6419	6543	L 6419	6431	753	6427	6497
week 6	R 6424[1])	6598	L 6424[1])	6435	750[1])	6408	6490[1])[3])
	R 6425	6541[1])	L 6425	6438[3])	751	6445	6491
week 7	R 6443[4])	6599	L 6443[4])	6436	6454	6446	6484
	R 6444	6540	L 6444	6448[4])	6455	6447[4])	6483[4])
month 3	6462	6539[1])	6441[1])	756	6548	763	6482[1])
	6467[1])	6537	6442	757	6561[1])	764	6485[1])
month 6	R 748	6473	740	L 748	6503	6501	6486[1])
	R 747	6536	L 747	741[1])	6504[1])	6502	6487[1])[3])
month 9			737				
			326				
month 12			328[1])				
			357				
month 24			358[1])				
			735				

Figures in chapter 4:

[1]) Photomicrograph histological section.
[2]) Macroangiogram.
[3]) Microangiogram.
[4]) Fluorescence photomicrograph.

operations: operations on the left femur formed group 3a, and operations on the right femur formed group 1. The animals were paired off for sacrifice 1, 2, 3, 4, 5, 6, 7 weeks and 3 and 6 months after operation. This means that for every survival period 2 animals per group were available for examination. Finally, 6 animals were kept alive until 9, 12 and 24 months after the operation (table 2). They were the 6 animals of the test group in which determination of intramedullary pressure had shown no rise due to leakage along the screw. It could therefore be assumed that no significant rise in intramedullary pressure had occurred. The acrylic cement used in these cases was Palacos (section 3.5.2).

3.7. *Sacrificing technique*

After intravenous injection of 50 mg (5000 IU) heparin, the animals were sacrificed by intravenous injection of an overdose of pentobarbital sodium (Nembutal). Immediately after cardiac arrest the abdominal cavity was opened and the aorta was cannulated at the level of the renal arteries with a plastic cannula inserted almost as far as the bifurcation. The aorta was ligated proximal to the site of cannulation. The inferior vena cava was opened. From a constant height of 100 cm (the most common physiological perfusion pressure) irrigation was effected with 1 litre physiological saline. The cannula was then connected with an infusion flask filled with a Micropaque® suspension.* This was a 6 per cent barium sulphate solution made from Micropaque 70 per cent W/W fine-granular barium sulphate and 35 g sodium citrate in 1 litre physiological saline. The first 300 ml of this solution could be run in quickly, the rest followed at a steady drip. As a rule, the suspension freely escaped from the inferior vena cava after about 1 minute. It took about 90 minutes for perfusion of the total amount of 1000 ml Micropaque suspension to be completed. The perfusion pressure was not measured. Next, both femurs were exarticulated in hip-joint and knee-joint, leaving an ample muscular cover in order to leave the periosteum intact. The bones were immediately numbered, packed in plastic bags and deep-frozen to –18° C.

3.8. *Radiological technique*

3.8.1. Radiographs during the period of observation

In the animals of the test group, radiographs of the pelvis and both femurs were made by a standard method before operation, immediately

* Micropaque® (Microtrast) Oesophageal Cream is supplied by Damancie & Cy Ltd, Slough (Bucks), Great Britain.

after operation and once a week thereafter. The purpose was to evaluate whether the method of cementing was sufficient (i.e. whether the entire femur was filled with cement) and to establish whether fractures had occurred during the operation or became manifest after the operation. In addition, it was expected that the femurs treated by operation would increase in circumference, as has been the case in the studies of Slooff (1970, 1971), who introduced acrylic cement into the medullary cavity in dogs. The radiological follow-up was made to establish whether the same could be observed in rabbit femurs.

3.8.1.1. Method:

The animal was kept still by intramuscular injection of 0.5 ml/kg Hypnorm (Philips Duphar) and 0.5 mg atropine, tied to a specially designed stand and submitted to AP radiography. The roentgen apparatus used was a Philips Super „Practix", and the film a Kodirex Autoprocess (no screen).

3.8.1.2. Results:

The medullary cavities of all femurs except two were entirely filled with acrylic cement. In one animal an avulsion fracture of the femoral diaphysis was observed which had been overlooked at operation. The mean increase in femoral circumference was small. A thin low-contrast layer of periosteal bone apposition was usually visible along the entire cortex from the second postoperative week on. The layer attained its maximum thickness after 3 weeks and, in the 14 animals with completely cement-filled medullary cavities, caused a mean increase in circumference of 9 per cent, as measured on a fixed point at the centre of the diaphysis. Subsequently the density of this new bone increased, and no further radiological changes occurred after 7 weeks.

3.8.1.3. Discussion:

The enormous bone apposition which Slooff (1970, 1971) observed in cemented dog femurs, was not found in rabbit femurs. Since the above described radiographic method was rather cumbersome, since the animals' biorhythm was being disturbed every week, and since the information obtained was of little value, these radiographs were not included in the ultimate experiment.

3.8.1.4. Conclusion:

As weekly radiographic follow-up failed to yield anything of value, it was abandoned for the animals in the ultimate experiment. Immediately after operation a radiograph was always obtained to make sure that the entire medullary cavity was filled with cement and to ascertain that no fracture had occurred.

3.8.2. Postmortem radiographs

To assess the degree of filling of vessels with Micropaque, radiographs were made in two directions of all deep-frozen femurs, using mammography equipment with a molybdenum anode. Very fine-grained industrial roentgen film was used so that enlargements were possible if required. This technique has been found very suitable for evaluation of bone structure (Meema et al. 1972; Hendriks 1975).

3.9. *Histological technique*

Of the deep-frozen femurs with soft-tissue lining, only the diaphysis and a small part of the lower methaphysis were used. This was manually cut into 7 transverse segments of about 0.75 cm thickness, which were numbered A, B, C and D from proximal to distal end. Segment C was bisected longitudinally, thus giving a total of 8 bone fragments. Of each segment A, B, C and D, one half was decalcified for paraffin processing and the other half was not decalcified but destined for embedding in plastic. The segments A, B and D were cut into transverse, and segment C into longitudinal sections (fig. 11). The procedure to obtain paraffin sections was: decalcification during 2 days in 5 per cent trichloracetic acid (which is also a fixative fluid which ensures persistent stainability), followed by washing with 90 per cent alcohol for 1 hour and then dehydration with 70 per cent alcohol, 80 per cent alcohol and 90 per cent alcohol and toluene (each during 24 hours), finally followed by degreasing with chloroform during 12 hours. Next, the tissue fragments were soaked in paraffin during 12 hours in an incubator at 60° C to ensure evaporation of the chloroform. The fragments were then embedded in paraffin and a Leitz microtome was used to cut 5 micra sections. These sections were stained with haematoxylin-eosin and according to Goldner (Romeis 1968; Schenk et al. 1969). The Goldner stain gives non-mineralized connective tissue (e.g. osteoid) an orange red colour; bone stains green, cells black, cytoplasm orange and muscular tissue reddish-violet.

The procedure to obtain the non-decalcified sections was: fixation in 70 per cent alcohol during about 16 hours, followed by dehydration with 80 per cent alcohol, 96 per cent alcohol, 100 per cent alcohol, acetone and toluene (each during 24 hours). The slices were then embedded in methylmethacrylate and, after curing, cut into 110 micra sections with a diamond section-cutting machine (instrument workshop, Department of Dentistry, University of Groningen). These sections were polished on both sides (Kent MK_2 polishing machine, Engis, Alphen a/d Rijn, Netherlands) until a thickness of 100 micra was attained. They were not stained. The non-

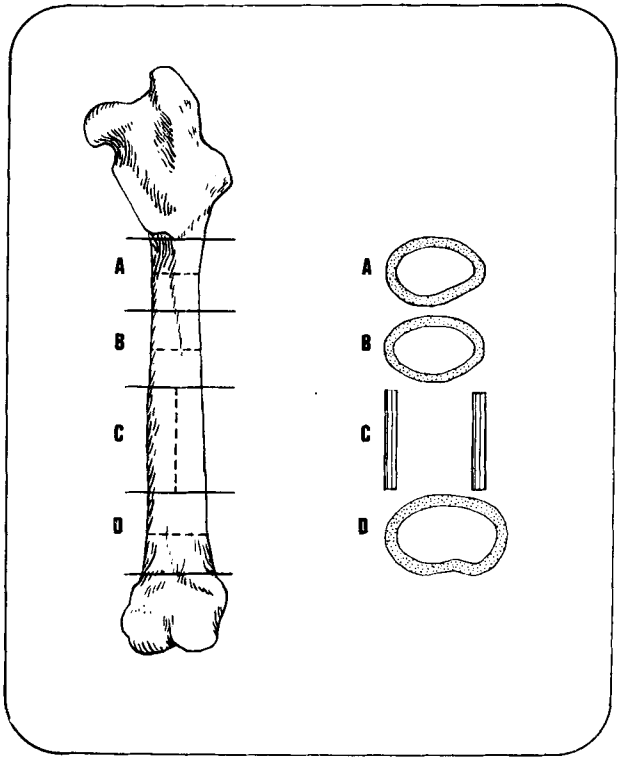


Fig. 11: Schematic representation of segments used for decalcified and non-decalcified sections.

decalcified sections thus obtained were embedded as a histological section with a non-fluorescent adhesive (DPX) and studied with the fluorescence microscope (section 3.11). The procedure described caused disappearance of acrylic cement from the bone by dissolution in trichloracetic acid, toluene, chloroform, xylol and acetone.

3.10. *Microangiography*

To obtain microangiograms of the non-decalcified sections from segments A, B, C and D of left and right femur, use was made of a microradiogram arrangement with a Philips self-rectifying X-ray generator type PW 1008 and Kodak spectroscopic safety film type 649-0 (20 min; 20 kV; 30 mA). The sections were placed directly on the film, the distance from the tube being 20 cm. The microradiograms obtained were placed on a slide and examined with the light microscope.

3.11. *Fluorochrome labelling*

Ordinary histological sections do no justice to the dynamics of such processes as growth, remodelling and regeneration of bone. The use of vital staining agents which are bound to newly formed bone of any origin (Harris 1960; Harris et al. 1964; Frost 1969) has added a new dimension to the study of these dynamic processes.

In addition to the bestknown fluorochromes, the tetracyclines (Milch et al. 1958; Harris 1960; Harris et al. 1962), a need has arisen for other vital staining agents to be used in sequential labelling (Olerud and Lorenzi 1970; Rahn 1973). Alizarin Red S is perhaps too toxic (Harris 1960; Adkins 1965). Moreover, it has been reported to inhibit bone formation (Harris et al. 1964), although others have refuted this (Vilmann 1969). Olerud and Lorenzi (1970) used it only immediately before sacrificing the test animal.

Fluorochromes for experimental use which have a low systemic toxicity and no local effect on calcification, and at the same time give clearly defined colour differences, are calcein (Suzuki and Mathews 1966), calcein blue (Rahn and Perren 1970), xylenol orange (Rahn and Perren 1971) and Alizarin-complexon (Rahn and Perren 1972).

During induction of anaesthesia, all the animals used in the experiment received an intravenous injection of 50 mg/kg oxytetracycline (Terra-

Table 3. Fluorochromes given for sequential labelling to the animals observed over a period of 7 weeks.

Fluorochrome	Dose per kg body weight	Mode of administration	Time of administration in weeks	Vertical illuminator III RS colour in	
				position 1	position 3
Terramycin	50 mg	i.v.	0	white	yellow
Xylenol orange	90 mg	s.c.	1	pink	orange
Calcein blue	30 mg	s.c.	2	blue	—
Alizarin-complexon	30 mg	s.c.	3	orange	deep red
Xylenol orange	90 mg	s.c.	4	pink	orange
Calcein	30 mg	s.c.	5	yellow	green
Alizarin-complexon	30 mg	s.c.	6	orange	deep red
Alizarin Red S	50 mg (total)	i.v.	7 (- 24 hrs)	pink	red

mycin® Pfizer); 24 hours before sacrifice they were given 50 mg Alizarin Red S as 1 per cent sterile solution, likewise by intravenous injection.

For the animals used for macro-autoradiography, no exception was made to this rule, in view of the possibility that Terramycin in the dose given could have a slight inhibitory effect of bone formation (Danckwardt-Lilliestrom 1969; Rahn 1973). The two animals which survived 7 weeks after operation in each of the groups 1 through 5, were in addition given weekly one of the above-mentioned newer fluorochromes as 3 per cent sterile solution (Rahn and Perren 1970, 1971, 1972) according to the plan shown in table 3.

To study and photograph the sections we used a modified Zeiss Universal microscope with fluorescence equipment, the vertical illuminator III RS and the attached camera LS-Matic (Verhofstad and Schrijer, 1975). Incident light excitation was used. In relation to the light direction, the sequence of the optical system used was as follows.

Light source	high-pressure mercury vapour lamp OSRAM HBO 200 W/4 heat absorption filter KG 1 red absorption filter BG 38
III RS (position 3) with: (blue light excitation)	excitation interference blue filter KP 500 excitation interference blue filter KP 490 dichroitic interference mirror FT 510 barrier filter LP 520
or III RS (position 1) with: (UV excitation)	UV-permeable black glass UG 1 dichroitic interference mirror FT 420 UV barrier filter LP 418
Objective	Neofluar 6.3/0.2

For the colour reproductions presented in chapter 4, only position 3 of the vertical illuminator III RS was used; the calcein blue, which fluoresced only in position 1, is therefore not visible in these reproductions (table 3). The colour as reproduced on the film used (daylight film Kodak HS 23° DIN Ektachrom EH 135) visually corresponded with the colour of the microscope image. Exposure times were 2-20 seconds.

3.12. *Macro-autoradiography*

Autoradiography shows the distribution of radioisotope-labelled compounds over the various tissues. Bone-seeking radioisotopes are deposited in new and incompletely mineralized osteons and make it possible qualitatively to demonstrate distribution patterns in bone and such processes as growth and repair. In combination with histological and fluorescence techniques, it can be used to differentiate between processes of bone degeneration and bone regeneration.

To put it simply, the image produced by the radioactivity from a sample on a photographic emulsion is an autoradiogram. On the developed film, the sites where the radioactive element is localized appear as dark areas. The Strontium ion used in this study so closely resembles

the calcium ion that it is incorporated in bone crystals in an identical way. Sr^{89} is a beta-emitter with a half-life of 51 days (McLean and Budy 1964).

The femurs of 10 rabbits (2 from each of the 5 principal groups) were macro-autoradiographically studied 1 and 4 weeks after operation. In 2 rabbits from groups 4a and 5, an autoradiogram was made after 4 months (table 4).

The survival of rabbits after injection of 80 μCi ^{89}Sr - $SrCl_2$ in aqueous solution (Amersham) was always 24 hours. Immediately after sacrificing the animals, both femurs were removed and immersed for 10 minutes in a chilling mixture of dry ice and isopentane (–65° C to –75° C). Until further processing the bones were stored in a freezer at –18° C. The bones were fixed by embedding them in a suspension of carboxymethylcellulose in water (5 per cent, medium viscosity, temperature 0° C). After embedding the bones the CMC suspension was frozen by chilling it to –15° C. The CMC bloc was mounted on the movable stage of a microtome (Jung K) placed in a freezer (–15° C). Sections of 30 micra thickness were fixed on tape (Scotch 810 3AW) during cutting. The strips of tape were then stretched on cardboard sheets. Before a film (Agfa-Gevaert Structurix D7) was placed on the fixed sections, it was dried in a freezer (–18° C) during 48 hours. The exposure time of the film was about 5 days.

Table 4. The animals used for Sr^{89} macro-autoradiography, classified according to type of operation and period of observation.

Rabbit no.	Group	Type of operation	Survival in weeks
6463	1	RS*	1
6464	1	RS	4
6559	2	RS + Palacos rod	1
6481	2	RS + Palacos rod	4
6456	3a	RS + Palacos	1
6457	3a	RS + Palacos	4
6458	4a	RS + Palacos without catalyst	1
6459	4a	RS + Palacos without catalyst	4
6560	5	RS + Sulfix-6 with CMC gel	1
6479	5	RS + Sulfix-6 with CMC gel	4
6564	4a	RS + Palacos without catalyst	16
6563	5	RS + Sulfix-6 with CMC gel	16

* RS = reaming and suction of medullary cavity.

3.13. *Disqualified animals*

A total of 19 animals (13.5 per cent) were disqualified from the ultimate experiment, in which 141 animals were used. Of these 19 animals, 8 showed a femoral shaft fracture which in 7 instances was noticed only on postmortem radiographs or at examination of the histological sections. In 3 animals the femurs proved to be insufficiently filled with acrylic cement. Four animals, all from group 4 (acrylic „cement" without catalyst), died during operation from histologically demonstrated fat embolism. Two animals died shortly after intravenous injection of Alizarin Red S, and two died from an intercurrent disease.

3.14. *Summary*

In 7 x 18 rabbits femurs the medullary cavity was reamed, a vent-hole was drilled and the medullary contents were removed by suction. In view of preliminary experience with a test group of 25 animals, the vent-hole was drilled as routine procedure.

The procedure was continued by filling the medullary cavity with acrylic cement. Dependent on the type of acrylic cement used, the animals were divided into 7 groups:

- — only reaming and suction (group 1);
- — insertion of a loose-fitting intramedullary Palacos rod (group 2);
- — filling of the medullary cavity with commercial acrylic cement (groups 3a and 3b);
- — filling with acrylic „cement" without catalyst (groups 4a and 4b);
- — filling with a modified acrylic cement: Sulfix-6 with CMC gel (group 5).

The animals were sacrificed in pairs 1, 2, 3, 4, 5, 6 and 7 weeks and 3 and 6 months after operation, and the lower limbs were perfused with a Micropaque suspension. The femurs were processed for radiological, histological, microangiographic and fluorescence-microscopic examination. The acrylic cement dissolved during processing of microscopic sections. Six animals in which commercial acrylic cement had been implanted were followed up over periods of 9, 12 and 24 months. Of 10 animals from each group (1, 2, 3a, 4a and 5), the femurs were used for macro-autoradiography after 1 or 4 weeks. In 2 animals from each of the two groups 4a and 5, an autoradiogram was made after 4 months.

CHAPTER 4

RESULTS

4.1. *Premises*

The typical reactions which occur in medullary cavity, cortex and periosteum following an intramedullary intervention have been described in detail in chapter 2. The same reproducible histological changes were observed also in our material, and need not be discussed in detail again in this chapter.

In this discussion of results, emphasis will be placed on a *comparison* of the changes observed in the various groups. In all cases sections of the treated femur were also compared with those of the control femur. Since no measurements were made, the observations are only graphic and descriptive, and not suitable for statistical analysis.

The histological sections provided the basis for the material studied. All other investigations were complementary and helped to confirm the observations made on the histological material. To avoid repetition, the entire histological material, including the fluorescence-microscopic findings, will be collectively discussed in sections 4.3 and 4.4. An exception will be made for the animals studied by sequential fluorochrome labelling during 7 weeks (sections 4.6 and 4.7).

In the Goldner-stained sections the distribution and intensity of stained osteoid varied from section to section. Sometimes the osteoid was stained in one animal but not in the other. On the whole, the data obtained provided too little constant information to warrant definite conclusions. A detailed discussion would be detrimental to the surveyability of this documentation.

It was also found that longitudinal sections C (fig. 11) supplied no new information; shortly after the start of the ultimate experiment, therefore, section C was used for making transverse sections.

Since no essential differences in the reaction pattern of cortex, medullary cavity and periosteum were observed in the material from groups 3A (commercial acrylic cement Palacos) and 3B (commercial acrylic cement Sulfix-6), the two groups will be discussed under the joint heading group 3: RS + PMMA. The same applies to groups 4A and 4B, which

will be discussed under the joint heading group 4: RS + PMMA without catalyst.

The photomicrographs shown are representative of the group and observation period concerned.

The photomicrographs of histological sections shown in this section depict haematoxylin-eosin (HE) stains. The photomicrographs were made with the Leitz Orthomat on Scientia film 50-B-65. A green filter 546 nm was used in the exposures. The periosteum is always shown at the top of the photograph, and details are viewed from the endosteal cortex side. The legends to the figures mention in succession: observation period, group, femur involved, rabbit number, segment number (A, B, C or D) and, if necessary, type of cement.
Pa (= group 3A: commercial Palacos)
Su (= group 3B: commercial Sulfix-6)
Pa–C (= group 4A: Palacos without catalyst)
Su–C (= group 4B: Sulfix-6 without catalyst)

To summarize: the groups to be discussed in the following sections will be referred to as:

group 1: reaming (R) and suction (S)
group 2: RS + Palacos Rod
group 3: RS + PMMA
group 4: RS + PMMA without catalyst
group 5: RS + modified Sulfix-6 with CMC gel

4.2. *Macroscopic findings*

Apart from a sligthly larger diameter as compared with the control bone, the deep-frozen femurs showed no relevant changes. Near the lateral femoral condyle at the site of the vent hole, some callus tissue was usually found. The distal cut surface was chosen in such a way that this callus tissue did not fall in the most distal sector (D). Once the femurs were cut into sections it was invariably found that the acrylic cement always filled the entire medullary cavity and was always fixed firmly to the bone, regardless of the type of cement used. This did not apply to the loose-fitting rods in group 2; these, however, were encapsulated by regenerated bone marrow.

4.3. *The normal histological features of the other femur used as control*

The normal histological features of the transverse section of the control femur show, throughout the specimen, osteons with vital osteocytes

Fig. 12: Normal features of the cortex – R 6561 B (HE x 40; detail x 95). A thin lamellar layer of endosteal bone is demarcated by a cement line from a central zone made up of primary and secondary osteons. Lamellated bone is also seen on the periosteal side, with several cement lines indiçating that the cortex has locally increased in diameter as a result of appositional growth. No woven bone is seen anywhere. A layer of regularly spaced flattened cells is seen in the endosteum. The periosteum consists of an inner osteogenic layer and an outer fibrous layer.

regularly distributed over the cortex (fig. 12). A layer of regularly spaced flattened cells is found in the endosteum. Since the microscopic architecture of a bone can be regarded as a cumulative product of its own growth and remodelling, the cortex itself shows at several sites of the diaphysis many varying combinations of the structural patterns (Enlow 1968). The trilaminar structure shown in fig. 12 is one of the variations which can be encountered in cortical diaphyseal bone, and cannot be described as a typical structural pattern. The figure has been chosen, however, because the diaphyseal cortex is usually made up of superposed zones of different types of bone tissue rather than of a single layer. The fluorescence sections show, moreover, that diaphyseal growth normally takes place in transverse direction by formation of lamellated bone on the periosteal surface, while endosteal resorption alternates with lamellated bone apposition (cf. section 4.6).

4.4. *Histological findings (combined with fluorescence-microscopy)*

Observation period: 1 week

In *group 1*, one-eighth to one-fourth of the endosteal cortex shows

signs of necrosis. The bone lacunae are often empty. The nuclei are sometimes swollen and the cement lines show a sharper circular definition around the bone lacunae, resembling an onion-skin configuration. Occasional resorption lacunae appear. The endosteum is usually lacking, but in a few places a thin new endosteal layer has been deposited. The latter is confirmed in the fluorescence section by the presence of Alizarin Red S on the endosteal surface. The periosteum is thickened and a thin subperiosteal layer of woven bone has been deposited, in which many Micropaque-filled vessels are visible.

The medullary cavity contains old blood which shows incipient organization. Dead fragments of bone, detached as a result of the drilling, are also found in the medullary cavity. New medullary Micropaque-filled blood vessels are already visible.

The sections of *group 2* show approximately the same features as those of group 1. A thin necrotic endosteal layer is again in evidence; the subperiosteal reaction is slightly more pronounced, and in the medullary cavity the intramedullary rod, the contours of which are clearly defined, is occasionally already surrounded by new bone marrow. There are new medullary vessels.

In *group 3* the cortical necrosis is much deeper and involves one-half to two-thirds of the cortex, counting from the endosteum. There are occasional areas of less extensive necrosis. Not one vital cell is left in the endosteum. A few smaller resorption lacunae are localized in the middle zone of the cortex. The periosteum has markedly increased in thickness and contains many Micropaque-filled vessels. The subperiosteal layer is considerably thicker than that in groups 1 and 2. The medullary cavity is translucent (the acrylic cement is dissolved).

In *group 4* the cortex is necrotic over nearly its entire width. Only a very narrow zone of the outer part of the cortex contains an occasional vital cell. The periosteum shows marked reactive thickening and is highly vascularized; the new layer of subperiosteal bone is about twice as thick as that in group 3. The medullary cavity is again translucent.

In *group 5* the endosteal part of the cortex again shows necrosis, but much less extensive than that in group 3 and certainly less than that in group 4. The features are more reminiscent of those in groups 1 and 2, but at some sites the necrosis is slightly more extensive. The subperiosteal reaction, too, is somewhat more pronounced than that in groups 1 and 2. Again there is marked vascularization. The medullary cavity contains fragments of dead bone and debris, no blood vessels being visible.

Observation period: 2 weeks

In *group 1* the extent of cortical necrosis is largely confined to a narrow endosteal zone. There are occasional areas of slightly deeper necrosis.

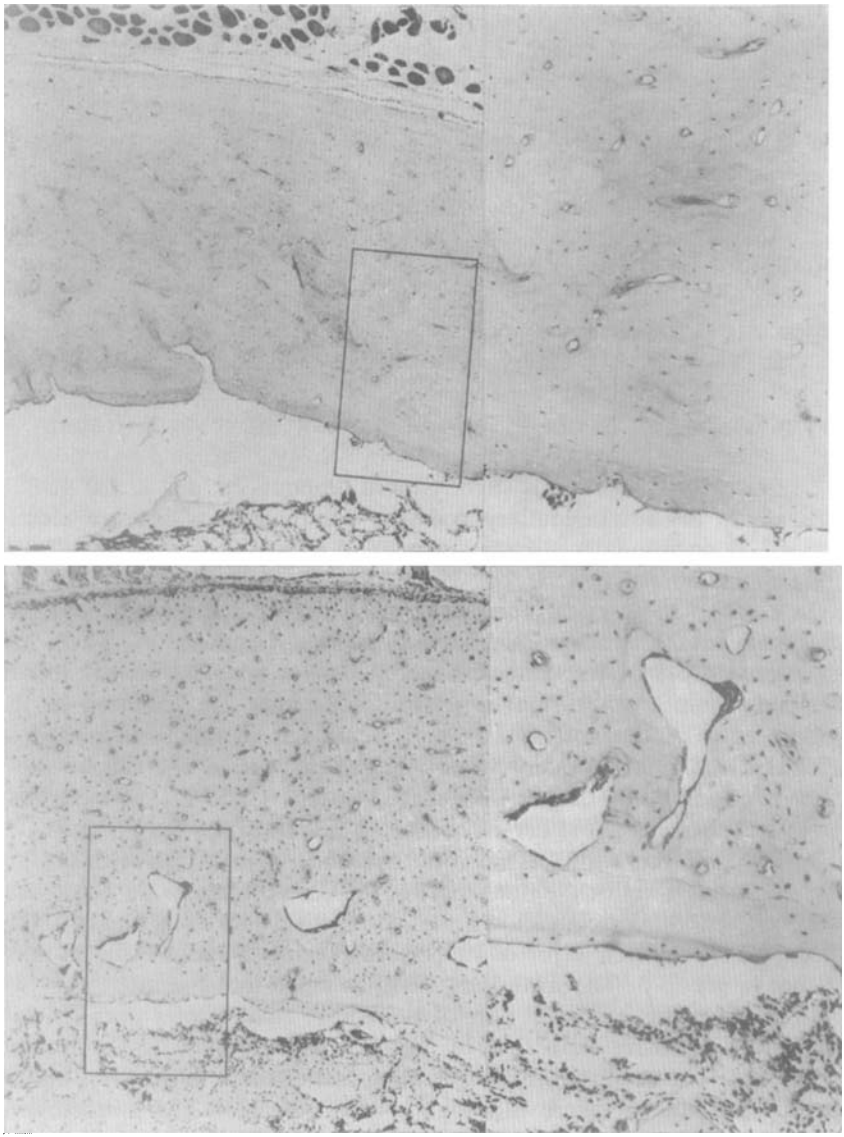


Fig. 13: 2 weeks, group 1 – R 6413 D (HE x 40; detail x 100). Hardly thickened periosteum; the new subperiosteal layer has been incorporated in the old cortex. Cortical necrosis is confined to a narrow endosteal layer. There is a new endosteum, which is forming bone (detail). In the medullary cavity regeneration of bone marrow elements and formation of bone spicules.

Fig. 14: 2 weeks, group 2 – L 6600 D (HE x 40; detail x 100). The features resemble those of group 1. Resorption lacunae are observed in the inner side of the cortex.

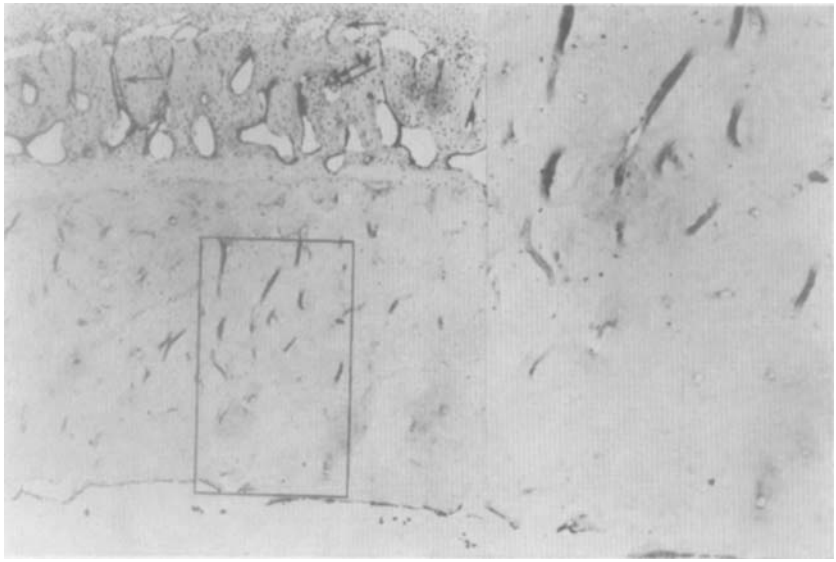


Fig. 15: 2 weeks, group 3 - L 6453 D - Su (HE x 40; detail x 85). Greatly thickened periosteum; thick, irregular layer of woven bone in which many Micropaque-filled blood vessels (arrows). At least two-thirds of the cortex is necrotic, but islets with vital osteocytes remain. No new endosteum is found (detail).

The endosteum shows resorption lacunae indicating removal of dead bone; new bone is being deposited around these areas. A new endosteum is present, from which young lamellated bone is being formed. The subperiosteal layer of woven bone shows a more mature appearance, and at most sites is incorporated in the old cortex (fig. 13). A new endosteal vascularization has developed in the medullary cavity, where pluripotent mesenchymal cells differentiate to bone marrow elements, but can also form small bone speculae. These are stained by the fluorochrome given last (Alizarin Red S).

The observations in *group 2* are almost identical (fig. 14). A connective tissue membrane has formed around the intramedullary rod.

In *group 3*, as in groups 1 and 2, the extent of the cortical necrosis after 2 weeks is clearly visible. At least two-thirds of the cortex is dead, and at some sites more. In the necrotic cortex, however, islets of vital tissue have remained intact. Resorption lacunae are occasionally visible at the boundary of the outer two-thirds and inner one-third. The endosteum has disappeared. There is still a marked subperiosteal reaction, and the periosteum itself has greatly increased in thickness. The medullary cavity is translucent (fig. 15).

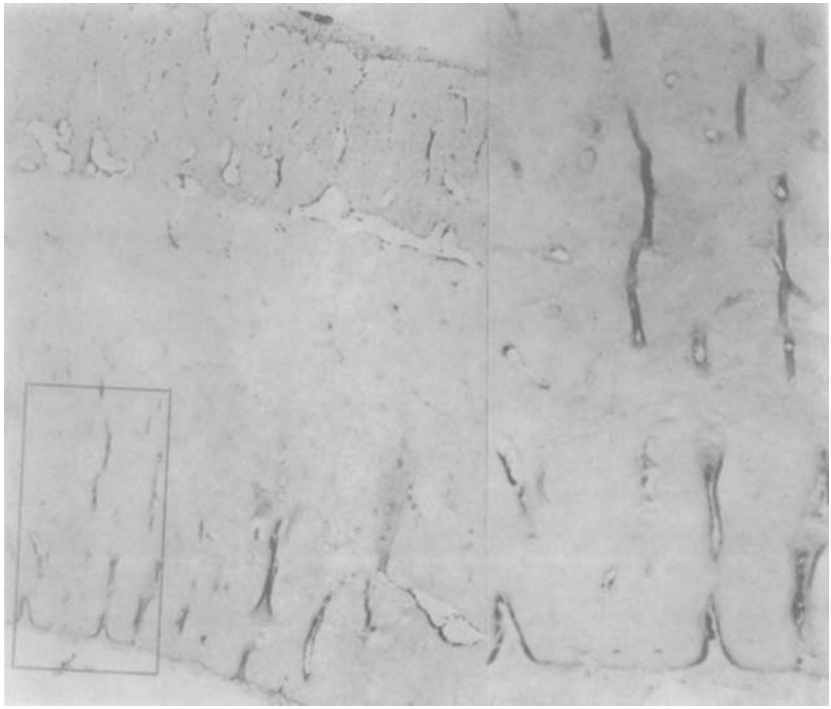


Fig. 16: 2 weeks, group 4 – L 6405 B - Su–C (HE x 32; detail x 110). Highly vascularized periosteum greatly increased in thickness; at some sites external callus has almost twice the thickness of the old cortex. Deep necrosis of nearly the entire cortex. No islets with vital cells are observed (detail).

In *group 4* the cortical necrosis is most extensive. Only an exceedingly narrow zone of cellular tissue has remained at the outer margin of the cortex. On the outside there is a thick layer of new woven bone, which at some sites has the same diameter as the old cortex. The necrosis is of much more „intensive" character because islets of vital tissue are not found anywhere in the dead cortex, as they were in group 3 (fig. 16). Implantation of acrylic „cement" with an overdose of monomer proves to cause the most radical damage of cortical bone tissue.

The sections in *group 5* are most reminiscent of those in groups 1 and 2. The necrosis is confined to the inner one-third of the cortex, but ample cellularized areas are found here. At most sites new endosteum has been formed, which contributes to endosteal osteogenesis. The thin layer of new subperiosteal bone is incorporated in the old cortex. The medullary cavity contains dead bone fragments and debris (fig. 17). This material must be localized in the pores of the modified acrylic cement.

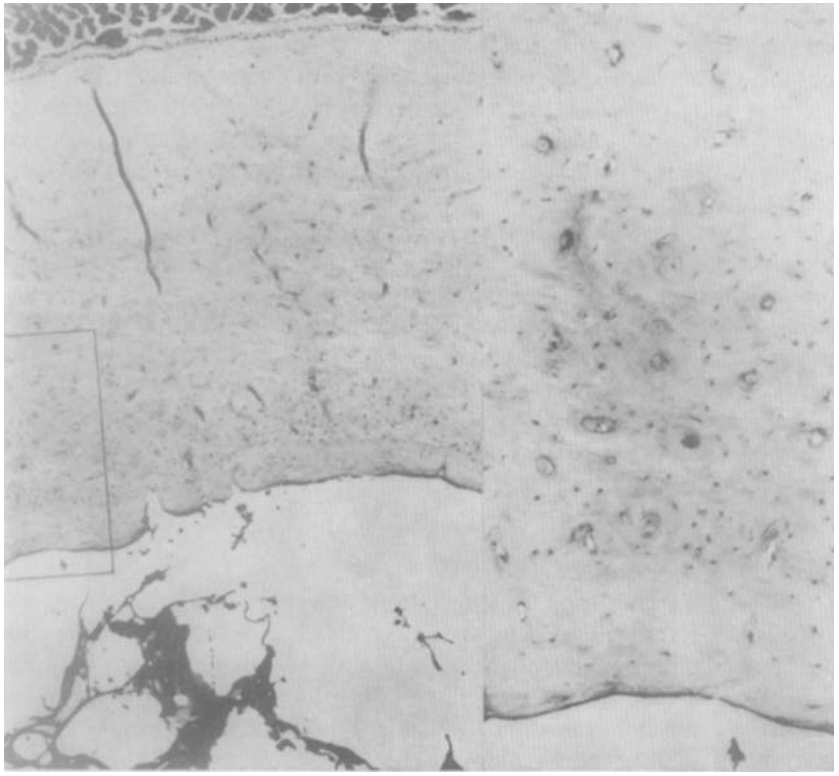


Fig. 17: 2 weeks, group 5 – L 6476 B (HE x 40; detail x 120). Cortical necrosis sligthly more extensive than that in groups 1 and 2, but features are nevertheless very similar. Dead bone fragments and debris are visible in the medullary cavity, i.e. in the pores of the cement.

Occasionally new medullary blood vessels can be seen pressed close against the cortex.

Observation period: 3 weeks

In *group 1* the necrosis of the inner cortex is actively resorbed; resorption lacunae increase in number and size, and new cellular bone is deposited around them. The new layer of endosteal bone, which is deposited in lamellae, has slightly increased in thickness. The vascularization in the periosteum has diminished to normal proportions. Numerous new blood vessels are present in the medullary cavity.
The sections in *group 2* show identical features.

In *group 3*, too, regeneration of the cortex has started to replace areas of necrosis. Resorption lacunae are still being found in the outer cortex,

usually at the boundary between two-thirds and one-third, but sometimes halfway. From the periosteum, tongues of cellular tissue invade the necrotic cortex and seek communication with islets of still vital bone. There is still a distinct line of demarcation between the subperiosteal bone layers and the old cortex. At a few sites the endosteum is beginning to restore itself. There is no connective tissue membrane between endosteum and acrylic cement.

In *group 4* large resorption lacunae are beginning to form in the outer layers of the necrotic cortex. The external callus is as thick as the old cortex, from which it is clearly demarcated.

In *group 5* resorption lacunae are likewise visible, but at the boundary between one-third and two-thirds, viewed from the inside. The cortical necrosis, which is slightly more extensive than that in groups 1 and 2, is being actively resorbed. The endosteal bone layer has substantially increased in thickness. Close against the cortex in the medullary cavity, many more new Micropaque-filled blood vessels are perceived than after 2 weeks' observation.

Observation period: 4 weeks

The sections in *group 1* show more advanced organization of the necrosis. The resorption lacunae remain small and lie close against the regenerating endosteal lamellae. At some sites, in fact, hardly any evidence of necrosis is left. In the medullary cavity the bone marrow shows an increasingly normal appearance, but many islets of bone persist.

In *group 2* the sections show no essential differences from those of group 1.

In *group 3* the organization of the cortical necrosis advances. Resorption lacunae are found in the inner cortical layers as well. At most sites there is new endosteum which, however, does not yet contribute to osteogenesis. The subperiosteal zone of new bone is maturing to lamellated bone and the demarcation from the old cortex is fading. The medullary cavity remains translucent; specifically, no new vessels are seen and there is no connective tissue membrane between endosteal cortex and acrylic cement surface.

In *group 4* elongated resorption lacunae are localized at the boundary between old and „new" cortex. The latter has matured, lamellated bone having replaced virtually all the woven bone. In the dead cortex, occasional vital osteocytes are only seen around resorption lacunae of ever-increasing size. There is as yet no endosteum and the medullary cavity remains translucent.

The sections in *group 5* show very active regeneration of the necrotic cortex by formation of resorption lacunae in the inner cortex and by „creeping substitution" from the inner and middle cortical layers. The layer of new endosteal bone increases in thickness, and the number of

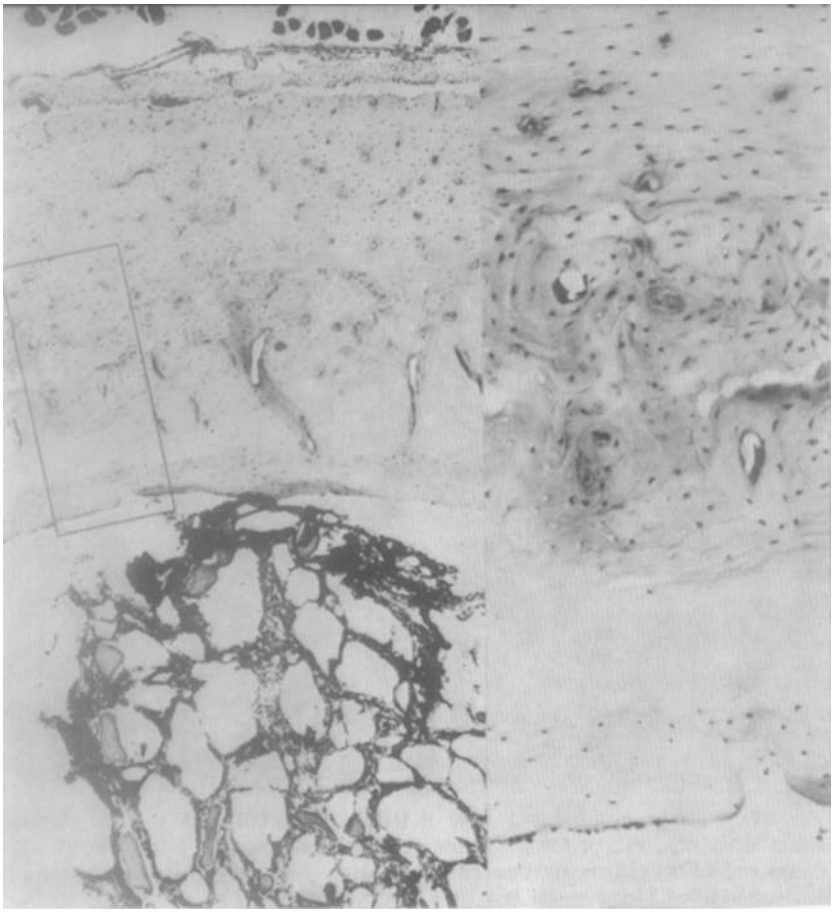


Fig. 18: 6 weeks, group 1 – R 6424 C (HE x 40; detail x 128). On the endosteal side of the cortex there is still a narrow zone of dead bone, demarcated from the medullary cavity by a new layer of lamellated bone. In the medullary cavity young bone tissue is visible there deposited next to bone marrow elements during regeneration.

blood vessels in the medullary cavity also shows some increase. An occasional vessel is observed in the centre of the medullary cavity, but most vessels are still localized close to the endosteum. Particles of contrast medium (zirconium dioxide) are observed in the medullary cavity.

Observation period: 5 weeks

The above-mentioned processes are advancing in each of the 5 groups.

In *groups 1 and 2* the amount of bone localized in the medullary cavity increases.

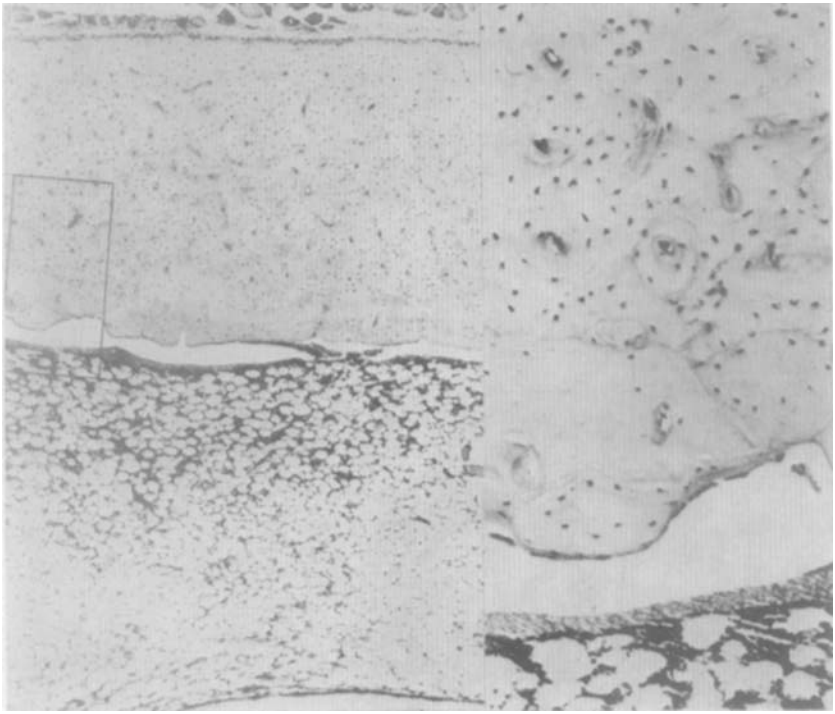


Fig. 19: 6 weeks, group 2 – L 6541 B (HE x 35; detail x 128). Nearly normal features of cortex, except for a narrow necrotic zone. Normal bone marrow. The Palacos rod (dissolved) is surrounded by a narrow connective tissue membrane. A Micropaque-filled blood vessel is visible in the periosteum.

In *group 3* the regenerated endosteum begins to form a thin layer of lamellated bone. At occasional sites fragments of the necrotic cortex are cast off into the medullary cavity as sequestra, and replaced by vital bone marrow.

In *group 4*, too, there is sequestration of necrotic endosteal cortex to the medullary cavity. New endosteum is still largely unformed. The necrosis has so weakened the bone that it readily ruptures when sections are being cut. The subperiosteal layer of new bone is not incorporated in the cortex.

In *group 5* the most striking feature is the increase in the number of medullary vessels, which are now localized also in the centre of the medullary structures must be localized in pores of the modified acrylic cement). In a number of specimens (rabbit 6497 B and D), even intramedullary bone is observed.

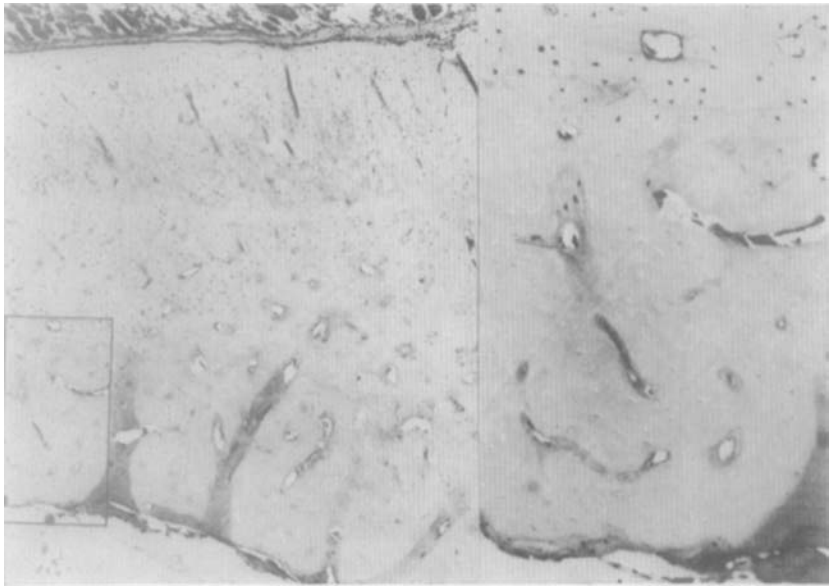


Fig. 20: 6 weeks, group 3 – L 6424 C - Pa (HE x 35; detail x 105). Still necrosis of one-half to two-thirds of the cortex, but active regeneration is in progress. Tongues of vital tissue grow into the necrotic cortex from the outside. New bone is being deposited around resorption lacunae. There is modest endosteal bone apposition. The subperiosteal bone layer has matured but is still distinguishable from the old cortex.

Observation period: 6 weeks

In *group 1*, all that remains of the necrotic cortex is a narrow zone on the inside, which is demarcated from the medullary cavity by a layer of newly formed bone. At some sites a large amount of bone is still seen in the medullary cavity, which shows abundant vascularization (fig. 18).

The sections in *group 2* show identical features, the bone marrow presenting quite a normal appearance (fig. 19).

In *group 3* half the cortex still shows necrosis, and active regeneration of new bone takes place. Otherwise there are few changes (fig. 20).

Group 4 shows but little progress in the restoration of the extensive necrosis. No new endosteal bone has yet been formed (fig. 21).

Group 5 again shows a close resemblance to groups 1 and 2. The necrotic layer on the inside of the cortex is slightly wider, however (fig. 22). A very interesting observation is made in the sections from both animals in group 5. New bone is localized in the centre of the medullary cavity (fig. 23)! It is well-vascularized, as is demonstrated by many small Micropaque-filled vessels found in and around this bone. At some sites

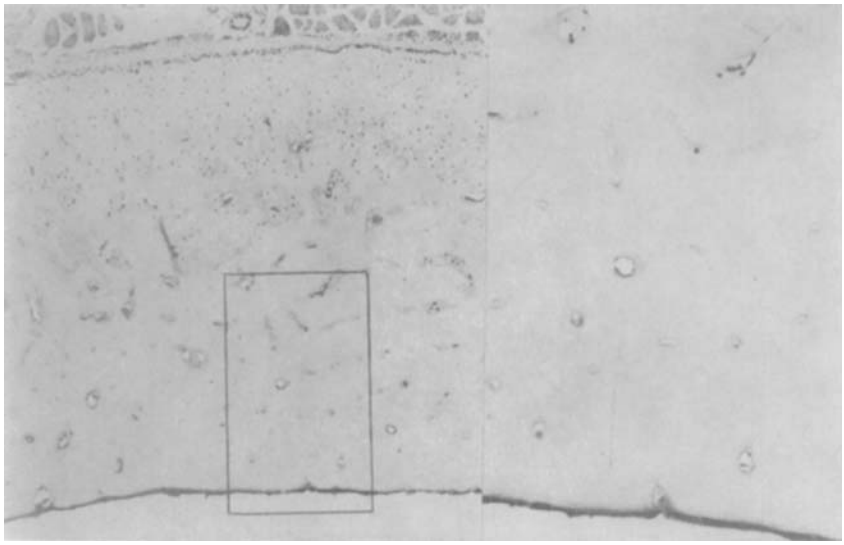


Fig. 21: 6 weeks, group 4 – L 750 D-Pa–C (HE x 40; detail x 100). By far the most severe necrosis. Islets of new bone are being formed from the subperiosteal layer. There is hardly any endosteal regeneration and no endosteal bone apposition is observed.

it is localized against small dead bone fragments. The new intramedullary bone is often localized in streaks of vital bone marrow. All these intramedullary structures must be localized in pores of the modified acrylic cement. This, however, has dissolved due to the processing of the material. But there remain particles of contrast medium which are trapped in the intramedullary structures (fig. 23). These particles are birefringent in polarized light. They are only very rarely seen in groups 2, 3 and 4, where the cement is solid.

Observation period: 7 weeks

In *group 1* an occasional endosteal necrotic area is still seen, but the cortex has been restored for the greater part. The above described new bone in the medullary cavity has disappeared, to be replaced by normal bone marrow elements which are more abundantly vascularized than in the control sections.

The sections in *group 2* show the same features as those in group 1.

Group 3 shows evidence of further organization and resorption of the necrosis. The resorption lacunae become slightly larger and some bone marrow sometimes appears in them. In some cases a very thin layer of connective tissue is seen along the new endosteum.

In *group 4*, new endosteum is seen in only an occasional specimen; it is usually still absent. The features of a deep inert necrosis of nearly

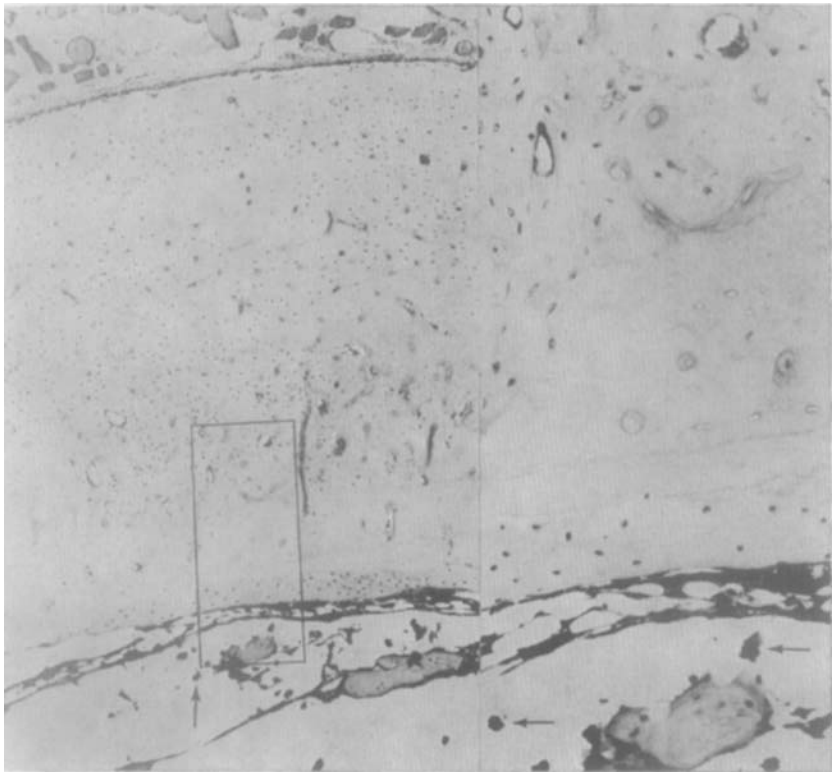


Fig. 22: 6 weeks, group 5 – L 6490 B (HE x 40; detail x 128). Features resemble those shown in figures 18 and 19, but the necrotic zone is slightly thicker. Pronounced layer of endosteal bone apposition. Strands of bone marrow and young bone in the medullary cavity. As remnants of the dissolved acrylic cement, contrast medium particles have remained intact (arrows).

the entire old cortex continue to dominate. Only the resorption lacunae increase in size and are now seen also on the inside of the cortex. Some are filled with bone marrow. On the inside of the cortex, dead bone slivers are occasionally cast off into the medullary cavity.

In *group 5* the necrosis also shows a considerable degree of organization, but less advanced than that in groups 1 and 2. Bone and bone marrow grow into the cement pores resembling the changes occurring after 6 weeks. This is observed in both animals and in all sections of segments A through D.

Observation period: 3 months

In *group 1* all sections from both animals show largely normal features.

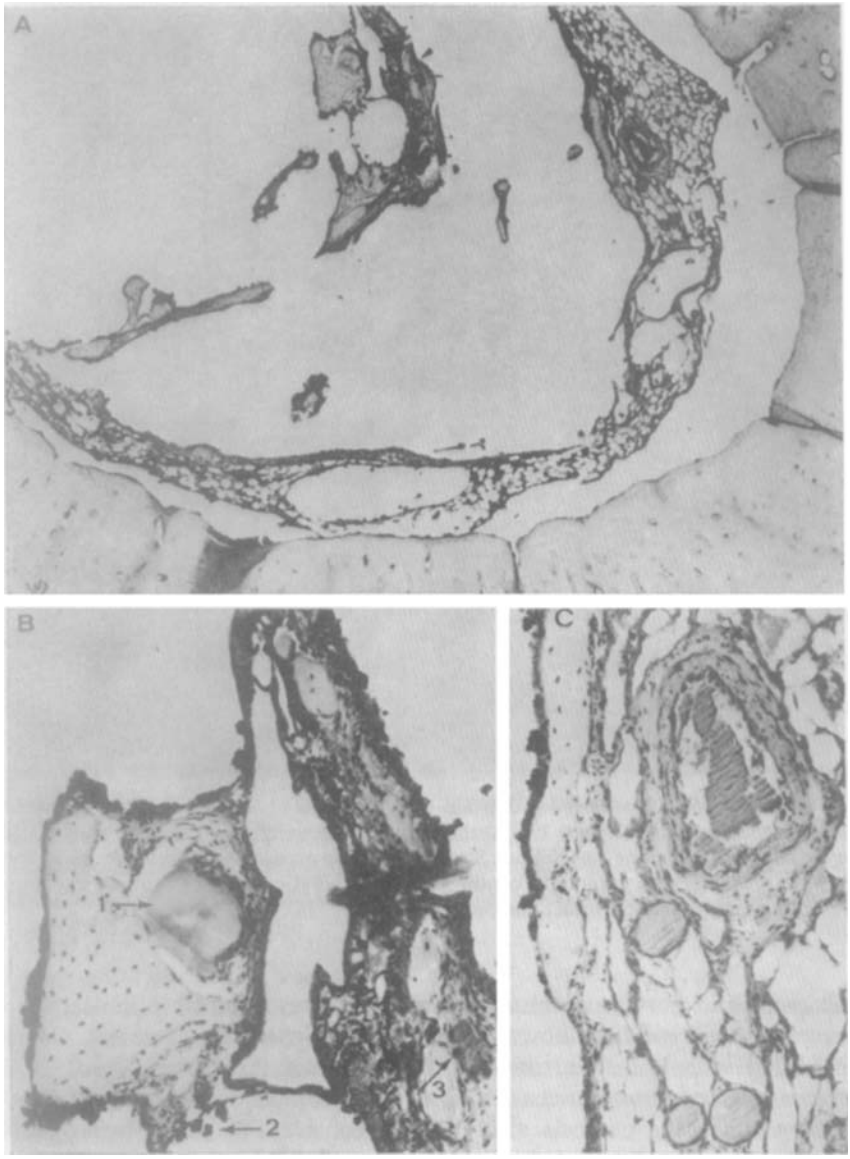


Fig. 23: 6 weeks, group 5 – L 6490 B (HE x 25 (A); detail x 110 (B); detail x 110 (C)). Strands of regenerated bone marrow and bone islets in the pores of the modified acrylic cement. Very active medullary revascularization (fig. 23A; fig. 23C). New bone (fig. 23B) can be deposited near dead bone fragments detached during reaming (arrow 1). Contrast medium particles are caught by the new intramedullary structures (arrow 2). The bone tissue in the pores is well-vascularized by Micropaque-filled vessels (arrow 3). Detail of the Micropaque-filled artery and arterioles localized next to a bone spicule (fig. 23C).

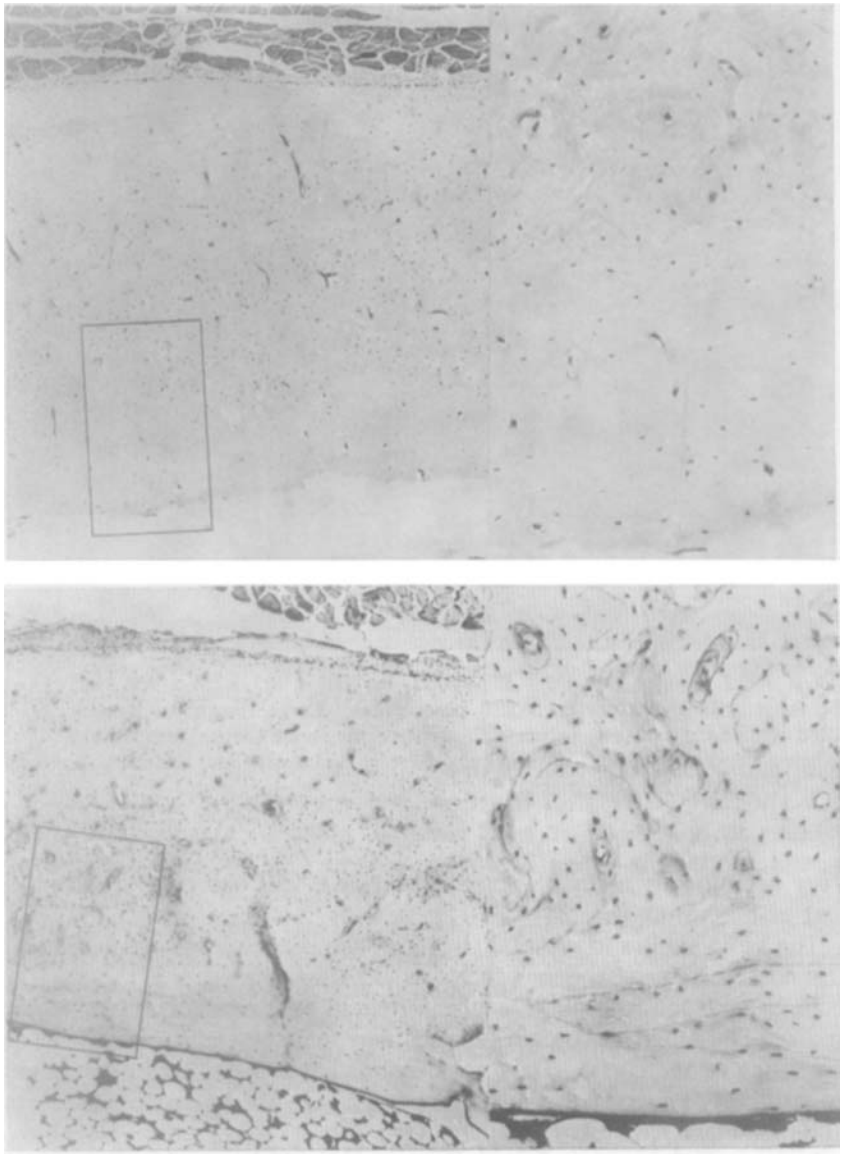


Fig. 24: 3 months, group 1 – L 6467 D (HE x 40; detail x 110). Virtually normal features of the cortex.

Fig. 25: 3 months, group 2 – L 6539 D (HE x 40; detail x 117). Normal features of the cortex.

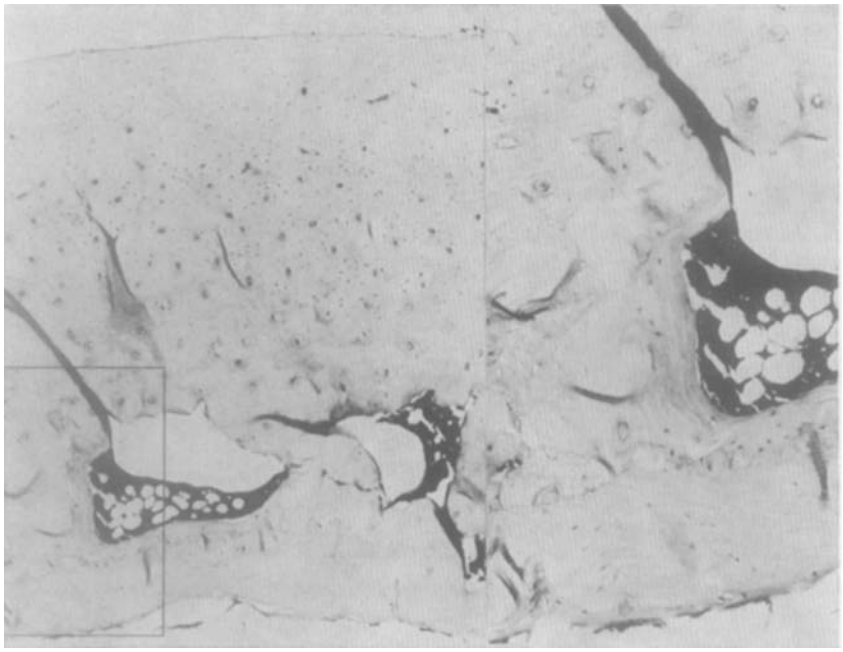


Fig. 26: 3 months, group 3 – L 6441 D - Pa (HE x 40; detail x 90). Necrosis of the inner half of the cortex is still predominant. Large resorption lacunae, often filled with bone marrow, in necrotic areas of the inner cortical layers. Vital bone is being deposited around these lacunae. Intact endosteum and endosteal bone apposition increased in thickness.

Very occasionally a small focus of necrosis is still observed in the inner part of the cortex (fig. 24).

The same applies to *group 2* (fig. 25). The connective tissue membrane around the Palacos rod has increased in thickness.

In *group 3* the resorption lacunae prove to have considerably increased in size. The majority contain normal bone marrow and are localized on the inside of the cortex. They are demarcated from the medullary cavity by a thicker layer of lamellated endosteal bone. The endosteum is intact. The medullary cavity is translucent (fig. 26).

In *group 4* the extent of cortical necrosis is still impressive. Resorption lacunae are seen throughout the cortex; a few of them contain bone marrow. Most of these lacunae are the centre of islets of highly cellular bone tissue. The subperiosteally appositioned bone has matured and been incorporated in the cortex. The endosteum has recovered and deposits a thin layer of endosteal bone. The medullary cavity remains translucent (fig. 27).

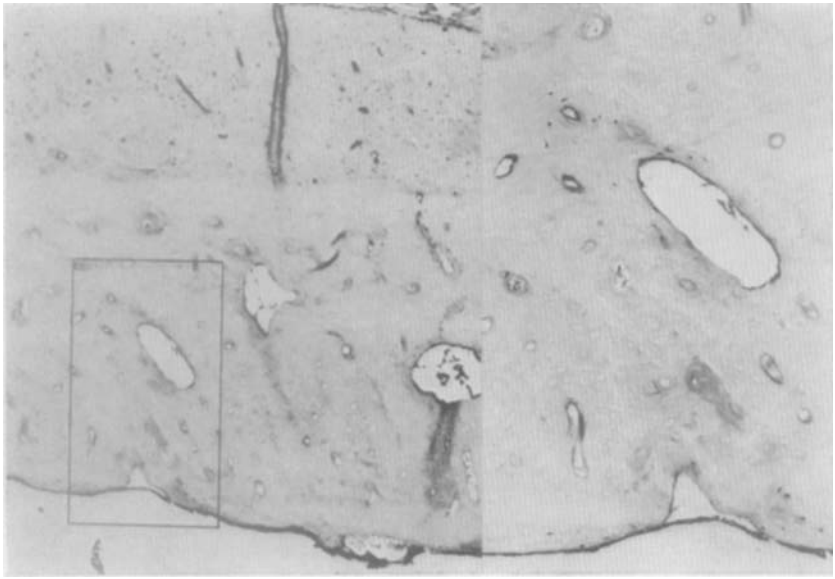


Fig. 27: 3 months, group 4 – L 6561 B - Pa–C (HE x 40; detail x 92). Very extensive cortical necrosis. Resorption lacunae throughout the cortex serve as centres for islets of vital bone tissue. The endosteum has regenerated and a thin zone of bone is being endosteally deposited.

In *group 5* the cortical necrosis in the inner zone is less organized than in groups 1 and 2 but nevertheless is pushed further and further back. Due to increasing regeneration the bone gives an impression of vitality. Resorption lacunae of the size seen in group 3 and 4 are entirely absent (fig. 28).

The growth of bone into the cement pores has evidently increased since the observations of 6 to 7 weeks. The medullary cavity is traversed by whole bone bridges with ramifications in all directions (fig. 29). This is not an incidental finding but a constant feature in all sections of the treated femurs of both animals. The granules of contrast medium are seen as small black dots among the bone and bone marrow spikes. They are apparently retained by the in-grown tissue, while the acrylic cement itself dissolves as a result of the processing of the sections (fig. 30A). In polarized light these crystals are birefringent (fig. 30B).

Observation period: 6 months

In *groups 1 and 2*, cortical bone and bone marrow have completely recovered from the operative trauma and present a normal appearance.

In *groups 3 and 4*, the „cancellization" or „medullization" of the cortex has continued. Large resorption lacunae filled with bone marrow

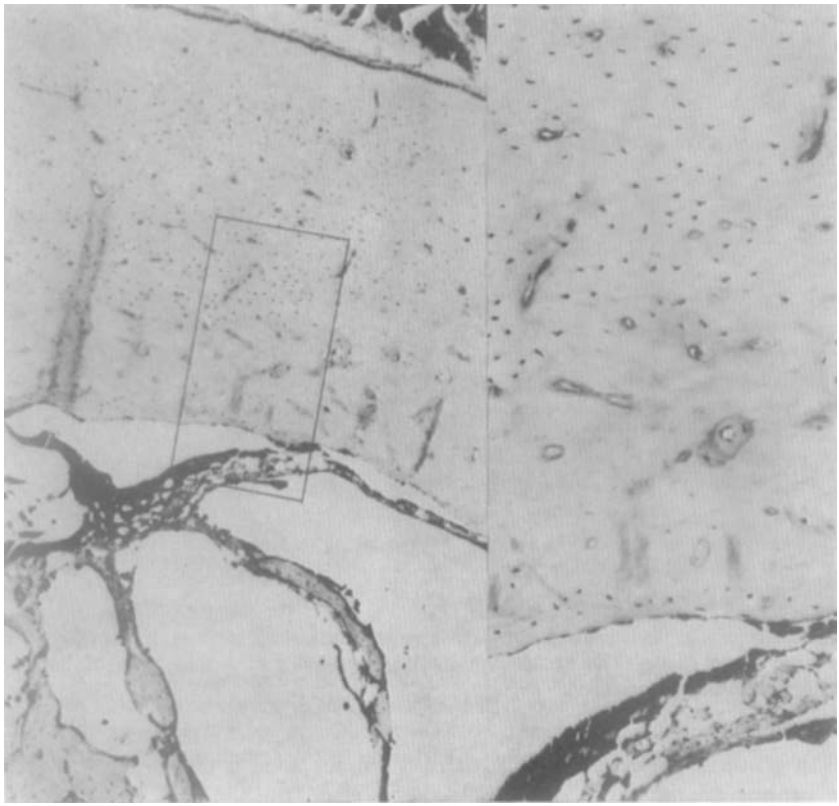


Fig. 28: 3 months, group 5 – L 6482 B (HE x 40; detail x 128). The necrosis in the inner cortical layer is being further resorbed and superseded. The cortex gives an impression of vitality. Growth of bone and bone marrow into the pores of the modified acrylic cement. Large resorption lacunae are not observed anywhere. This photomicrograph is a detail from fig. 29.

are localized in the necrotic inner half of the cortex (fig. 31, fig. 32). At some sites fragments of dead cortical bone are sequestrated into the medullary cavity (fig. 34). This is particularly evident in group 4. A thin connective tissue membrane is occasionally encountered in the interface between bone and acrylic cement. It can contain giant cells.

In *group 5* the necrosis of the inner cortex has practically disappeared (fig. 33), although an evidently poorly cellularized area is occasionally still seen. There are no large resorption lacunae as observed in groups 3 and 4. The new endosteal bone layer has for the greater part increased in thickness.

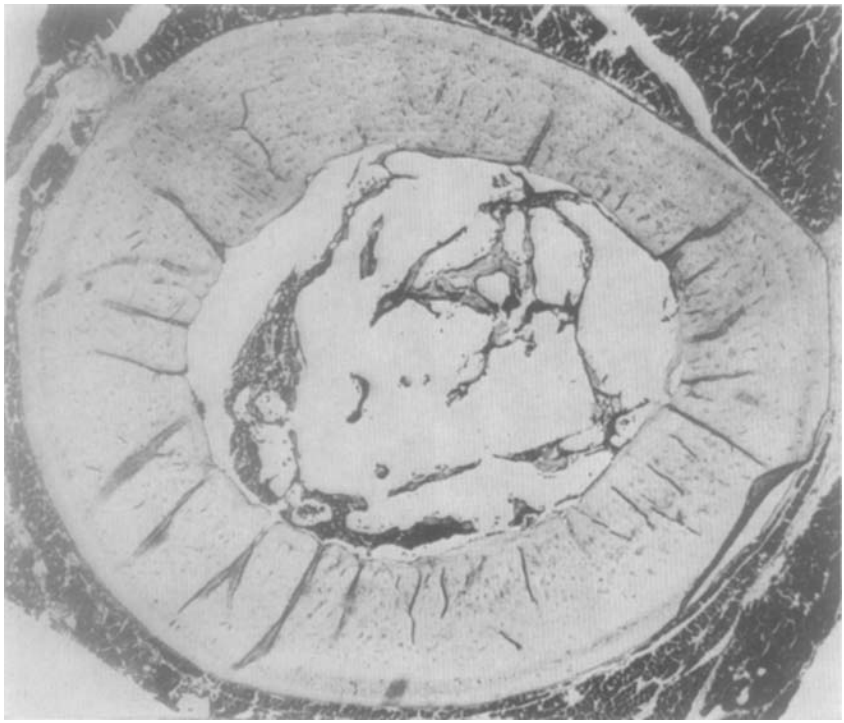


Fig. 29: 3 months, group 5 – L 6482 B (HE x 11). General view to demonstrate the intramedullary distribution of the bone and bone marrow tissue grown into the cement pores. Fig. 28 shows a detail of this section.

As compared with the features observed after 3 months, the intramedullary growth of bone and bone marrow into pores of the modified acrylic cement has increased. This is a constant feature in all sections from both animals (fig. 35A, fig. 35B).

Observation period: 9, 12 and 24 months

Only 6 animals of *group 3* (implantation of commercial acrylic cement) were followed up over a longer period of time (table 2).

After *9 months* there is a predominance of the previously described features of large resorption lacunae filled with bone marrow (medullization of the cortex) and sequestration of dead bone into the medullary cavity. Resorbed and sequestrated dead bone is replaced by bone marrow. Large necrotic areas are still seen in the inner half of the cortex. An unmistakable connective tissue membrane is seen in the interface between acrylic cement and bone.

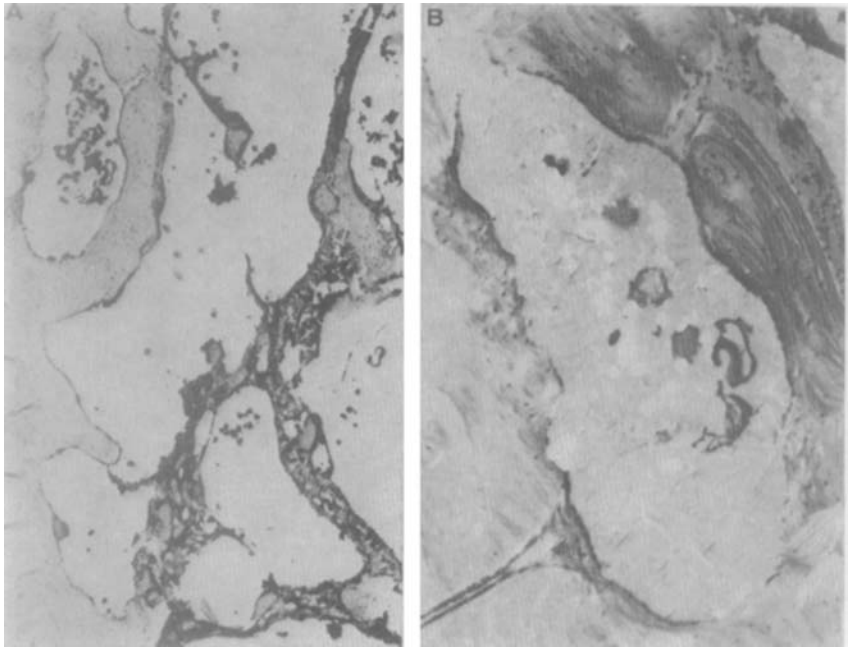


Fig. 30: 3 months, group 5 – L 6485 D (HE x 37; detail x 90). Granules of contrast medium (black dots) are enclosed by the bone grown into the pores of the cement. The development of this bony bridge could be exactly followed by a study of consecutive sections (fig. 30A). In polarized light the birefringent crystals of contrast medium stand out white. The bone trabeculae growing together show the structure of young bone (fig. 30B).

After *12 months* there are no essential further changes (fig. 36).

After *24 months* all dead tissue has been replaced by vital bone or resorbed. The acrylic cement is bounded by a thick connective tissue membrane which can contain giant cells. The entire necrotic inner half of the cortex has been replaced by bone marrow. In the remaining cortex, which consists of the old outer half of the cortex and the newly formed subperiosteal bone, dead tissue is no longer encountered (fig. 37).

4.5. *Angiographic findings*

Although the Micropaque perfusion technique used was not always found to be optimal, the results obtained were of value as a supplement to the histological findings.

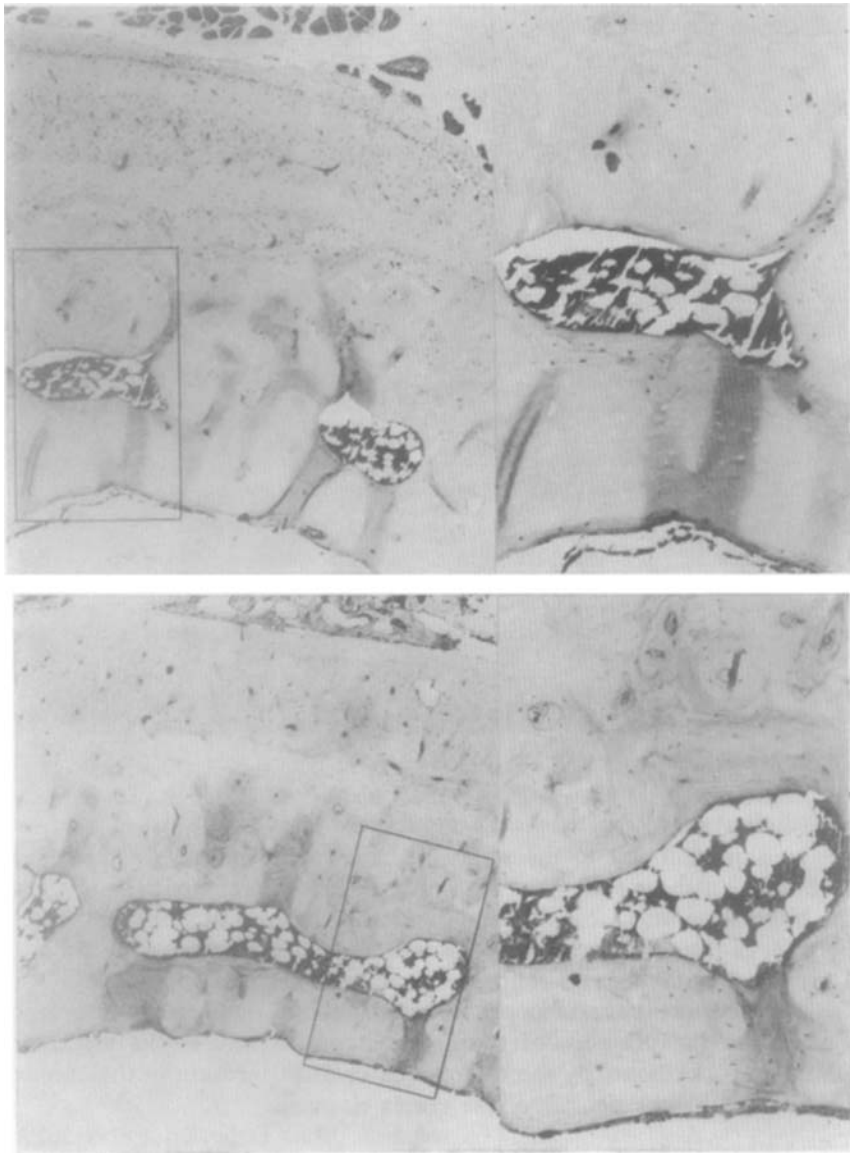


Fig. 31: 6 months, group 3 – L 741 D - Su (HE x 40; detail x 90). Medullization of the necrotic cortex. Large resorption lacunae filled with bone marrow in the inner half of the cortex.

Fig. 32: 6 months, group 4 – L 6504 B - Pa–C (HE x 40; detail x 90). Medullization in a cortex recovering from very extensive necrosis.

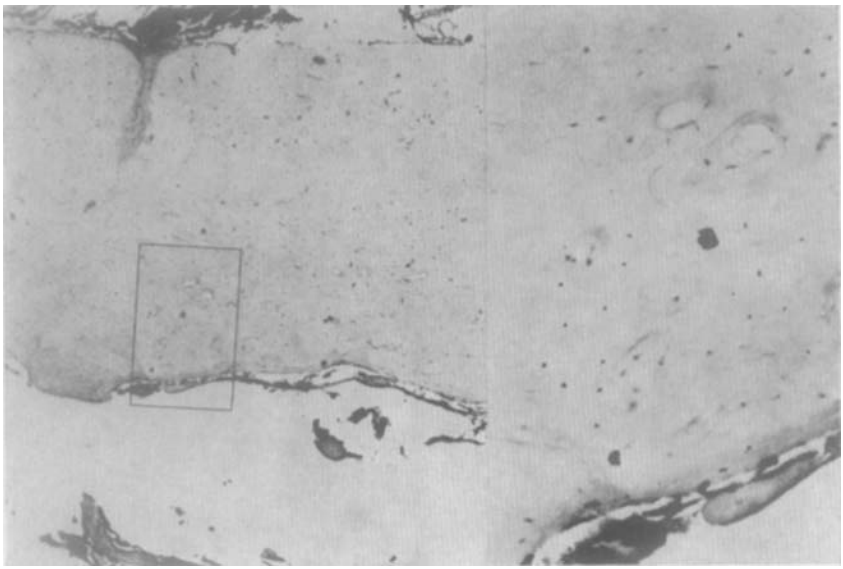


Fig. 33: 6 months, group 5 – L 6486 B (HE x 40; detail x 145). Virtually complete resorption of the necrosis of the inner cortex. Resorption lacunae of the size observed in groups 3 and 4 are not found anywhere.

4.5.1. Macro-angiography

In the untreated femur used as control, the entry of the nutrient artery was constantly found at the same site, immediately distal to the lesser trochanter on the medioposterior aspect of the femoral shaft (fig. 2A,B). The bifurcation into ascending and descending branches immediately after the passage through the nutrient foramen was likewise a constant finding. However, the number and calibre of these branches varied.

Evaluation of the macroscopic vascularization of the treated femur was impeded by the presence of the extra-osseous vessels in the muscular coat around the bone, by the radiopaque contrast medium in the acrylic cement, and by the density of the cortex as such.

Observation period: 1 week

In none of the 5 groups is a nutrient artery visible (fig. 38A, fig. 38B).

Observation period: 2-7 weeks

In *group 1* the typical course of the nutrient artery reappears after 2 weeks and shows nearly complete recovery after 5 weeks.

In *group 2* the course of the nutrient artery is of course influenced by

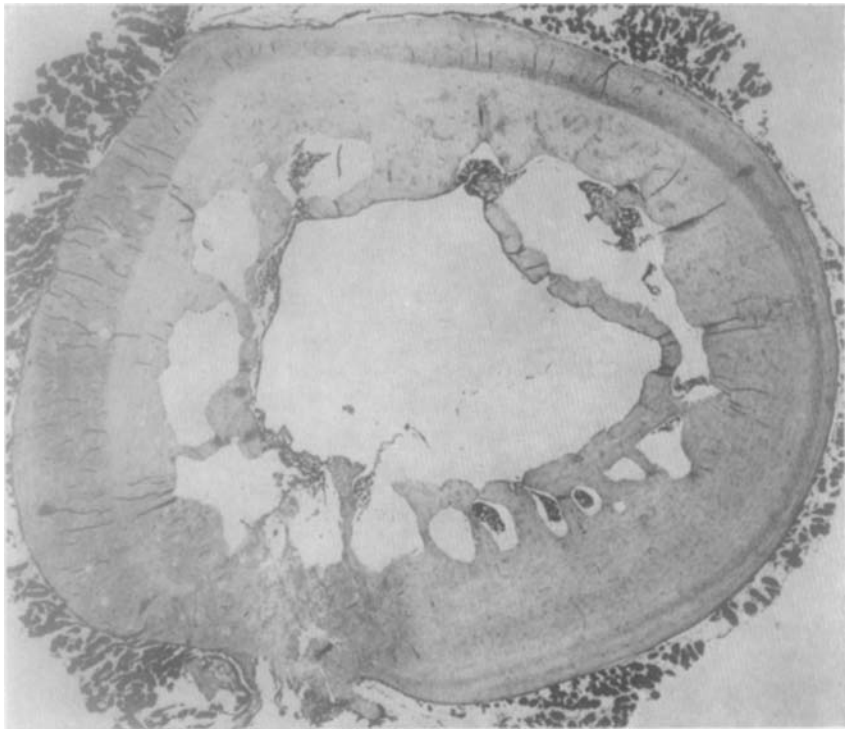


Fig. 34: 6 months, group 3 – L 741 C - Su (HE x 11). General view of „cancellization" or „medullization" of the cortex. Sequestration of dead bone into the medullary cavity. Thin connective tissue membrane in the bone/cement interface. The medullary cavity is translucent.

the intramedullary rod, but vessels are clearly seen to extend along the rod after 5 weeks (fig. 38C).

In *groups 3, 4 and 5*, as expected, the obliterated nutrient artery is nowhere to be seen after filling of the medullary cavity with acrylic cement.

Observation period: 3 and 6 months

In *group 1* the nutrient artery has returned to normal after 3 months.

In *group 2* the ascending and descending branches of the nutrient artery which extend along the rod, can be clearly differentiated after 3 months.

In *groups 3 and 4* there is nothing to suggest regeneration of arterial branches after 6 months.

In *group 5* an occasional Microtrast-filled vessel appears in the cement

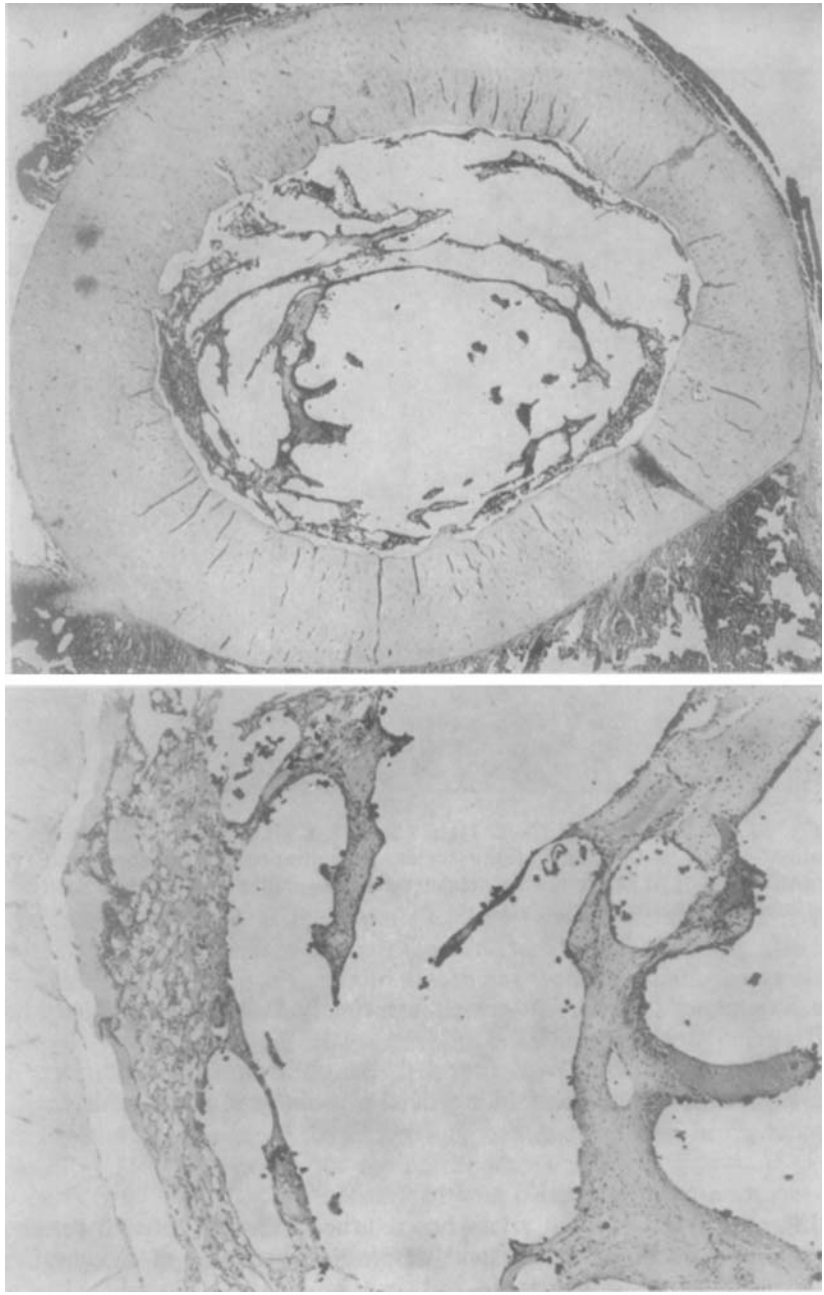


Fig. 35: 6 months, group 5 — L 6487 B (HE x 11; detail x 45). General view of the growth of tissue into the pores of the modified cement. Bone processes traverse the medullary cavity (fig. 35A) and can attain a considerable thickness (fig. 35B). As remnants of the acrylic cement dissolved in processing, occasional granules of contrast medium are encountered.

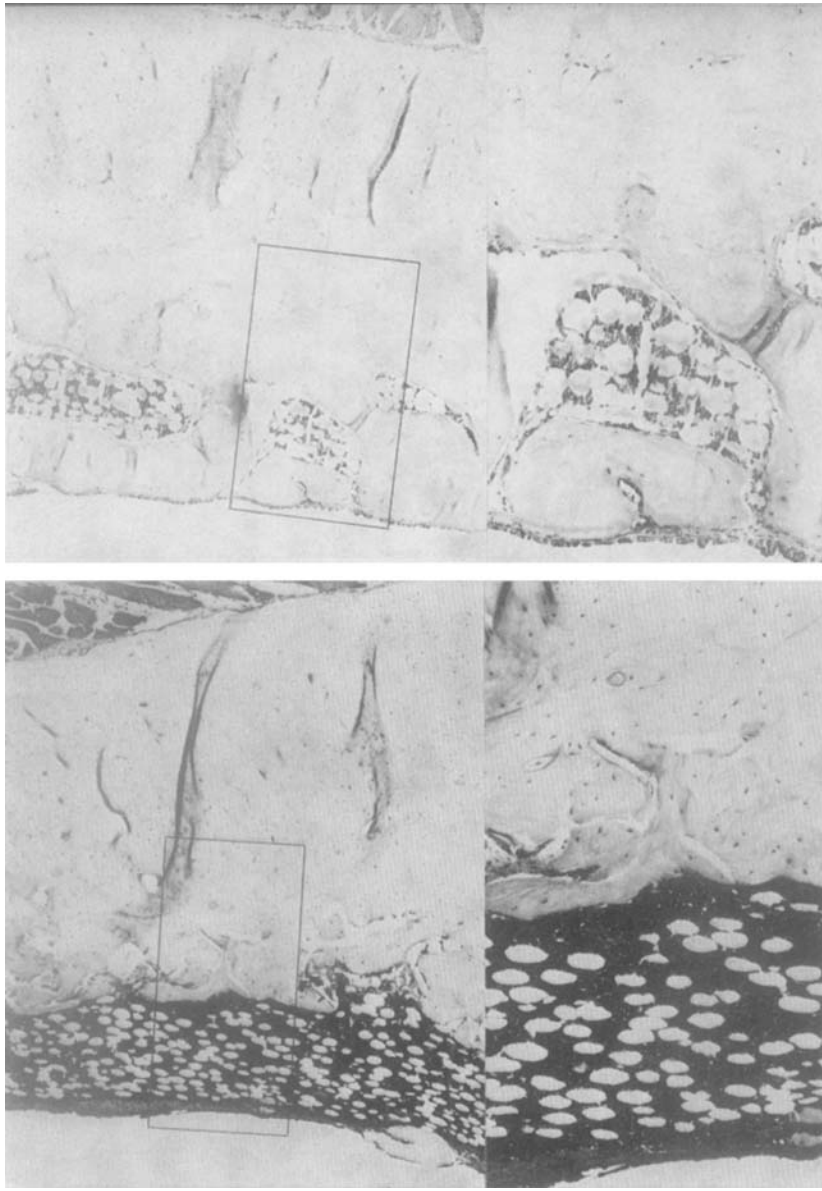


Fig. 36: 12 months, group 3 – L 328 D - Pa (HE x 40; detail x 92). Large resorption lacunae filled with bone marrow are seen in the necrotic inner half of the cortex. Necrotic bone fragments will be sequestrated into the medullary cavity. The remaining cortex still comprises many poorly cellularized areas. The transition to the subperiosteally apposed bone is still visible.

Fig. 37: 24 months, group 3 – L 358 B - Pa (HE x 40; detail x 100). The necrotic inner half of the cortex has been replaced by bone marrow and is separated from the acrylic cement by a thick connective tissue membrane. The remainder of the old cortex is vital but shows a somewhat erratic architecture.

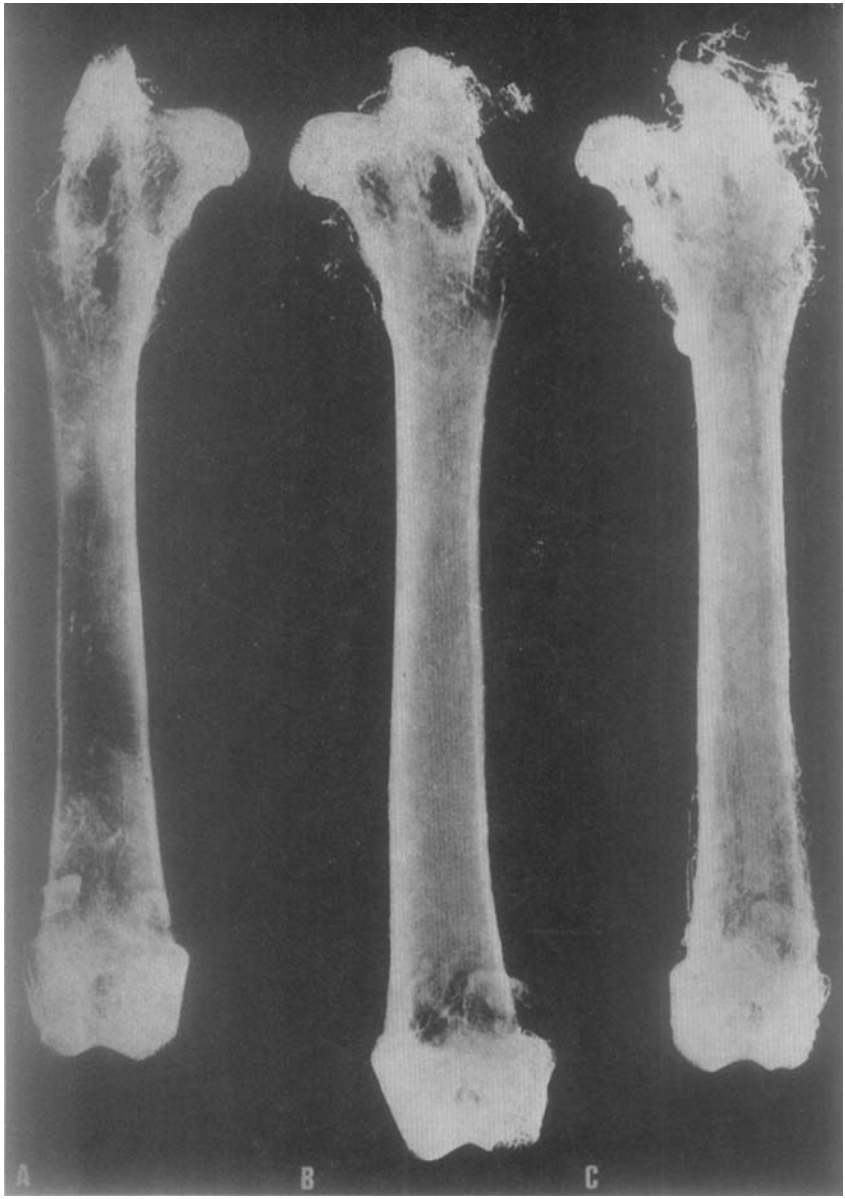


Fig. 38: Anteroposterior radiographs of rabbits femurs, using the mammography technique. In group 1 (R 6420) the nutrient artery is interrupted one week after reaming and suction of the medullary cavity. There are occasional signs of regeneration (A). In group 5 (L 6475) there is no longer any visible trace of the nutrient artery. The medullary cavity is filled with modified acrylic cement (B). In group 2 (L 6597), slender longitudinal vessels are seen to extend along the Palacos rod 5 weeks after reaming and suction of the medullary cavity and introduction of an intramedullary acrylic cement rod (C).

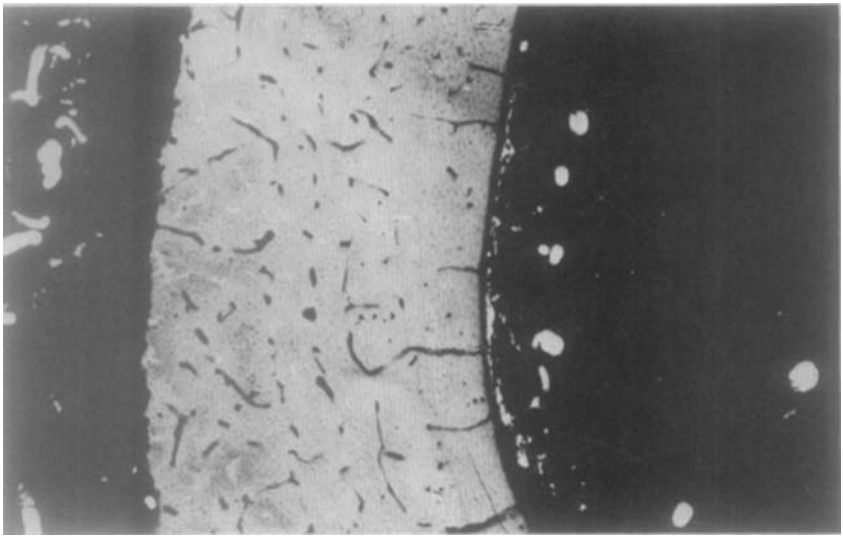


Fig. 39: The normal micro-angiogram of the control femur. R 6445 D (x 40). It is to be borne in mind that stimulated vascularization (Rhinelander 1972) may still be involved, because the left femur of the same animal was operated on 6 weeks earlier (table 2 - group 4b).

after 3 months, but this can be followed only over a distance of a few millimetres. After 6 months the features are more sharply defined. However, the possibility that extraosseous vessels extending in the muscular coat are involved, cannot be excluded with certainty.

Observation period: 9-24 months

In *group 3,* blood vessels extending longitudinally between acrylic cement and cortex can be observed from the 9th month on.

4.5.2. Micro-angiography

The *normal* micro-angiographic features of the control femur show the Micropaque-filled medullary arteries, while in the intracortical canals only an occasional small vessel fills with contrast medium. Extraosseous vessels around the cortex are likewise filled (fig. 39).

Observation period: 1 week

As the histological material also showed, numerous new blood vessels are already present in the medullary cavity in *group 1.* Vessels are seen also in the cortex, but in particular the subperiosteal zone of new bone is richly vascularized by blood vessels extending perpendicular to the cortex. This pattern of vascularization is also observed in *group 2.*

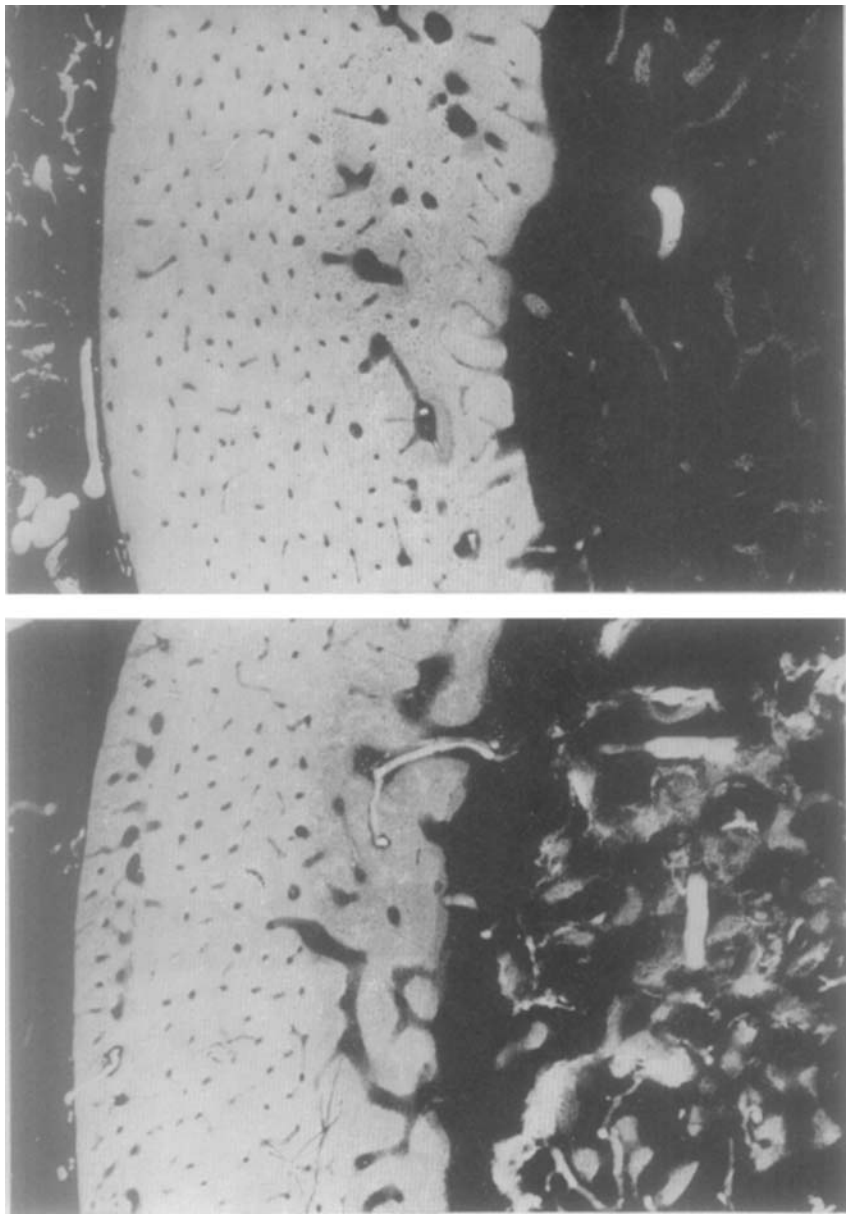


Fig. 40: 3 weeks, group 1 – R 6411 B (micro-angiogram x 40). Normalization of the pattern of vascularization of the cortex. Still unmistakable activity of extra-osseous vessels. New blood vessels and bone spicules in the medullary cavity.

Fig. 41: 3 weeks, group 2 – L 6545 D (micro-angiogram x 40). Virtually normal cortical vascularization. The zone of subperiosteally apposed bone is clearly identifiable. Very active regeneration of the medullary vessels with numerous bone spicules.

In *group 3*, no vessels are visible in medullary cavity and old cortex; only a thick subperiosteal layer of new bone is richly vascularized by extraosseous vessels which have substantially increased in calibre and number. The same features are seen in *group 4*, in which in particular the thickness of the layer of appositional bone is striking.

The micro-angiograms of *group 5* show a striking resemblance to those of groups 1 and 2, but no vessels are visible in the medullary cavity.

Observation period: 2-7 weeks

From the 2nd week on the medullary revascularization in *groups 1 and 2* increases enormously, these vessels being many times as large in number and calibre as those in the control femur. The medullary cavity contains bone spicules deposited there during regeneration of medullary contents. In the cortex the endosteal vascularization increases, the entire cortex being revascularized after 3 weeks. With the increase in medullary vessels vascular overfilling in the new subperiosteal bone ceases, and returns to normal proportions in the 3rd week (fig. 40, fig. 41).

In *groups 3 and 4* medullary revascularization takes a much slower course. Up to the 6th week, no new medullary vessels are observed; and it is not until after 5-6 weeks that reactive vascularization in the woven subperiosteal bone has normalized. Only after 4 weeks in *group 3* and after 6 weeks in *group 4* are sufficient endosteal vessels in the cortex observed to warrant the conclusion of a restored cortical vascularization. The impression is that cortical revascularization takes place from periosteum to endosteum in these two groups (fig. 42, fig. 43). After 6 weeks Micropaque-filled vessels are seen between cortex and acrylic cement in both groups (fig. 45).

Just as with the histological material, the micro-angiograms of *group 5* are most reminiscent of those in groups 1 and 2. From the 2nd week on, new blood vessels are observed in the medullary cavity, and Micropaque-filled vessels are seen endosteally at most sites in the cortex. Cortical vascularization has been restored after 3 weeks (fig. 44). Medullary vessels gradually increase in number, and after 6 weeks there is enormous proliferation of these new vessels and vascularized bone spicules, which must be localized in the pores of the cement, are observed (fig. 46).

Observation period: 3 and 6 months

At 3 months, cortical vascularization in all groups has returned to normal in the sense that the pattern of vascularization resembles that shown in fig. 39.

In *group 1* the vascularization of the medullary cavity has also normalized after 3 months. The same applies to *group 2*, although here space is occupied by the rod, surrounded by a vascularized connective tissue membrane.

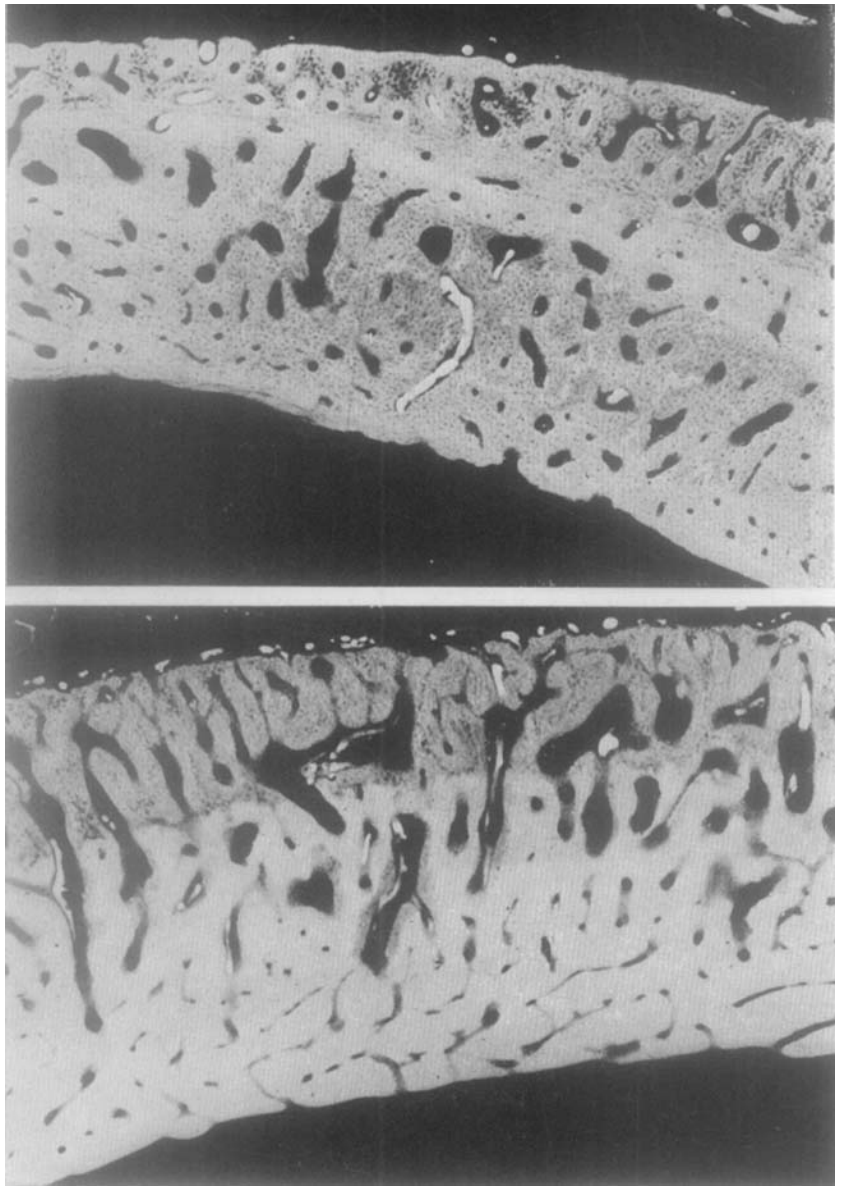


Fig. 42: 3 weeks, group 3 – L 6410 C - Pa (micro-angiogram x 40). Many Micropaque-filled blood vessels are visible particularly in the subperiosteal layer of new bone. Only an occasional vessel has filled in the endosteal part of the cortex. No blood vessels in the medullary cavity.

Fig. 43: 3 weeks, group 4 – L 6414 D - Su–C (micro-angiogram x 40). Marked vascular activity in the subperiosteal bone layer; the impression is that the cortex is being revascularized from this layer. As yet no Micropaque-filled vessels in the inner part of cortex. The medullary cavity is translucent.

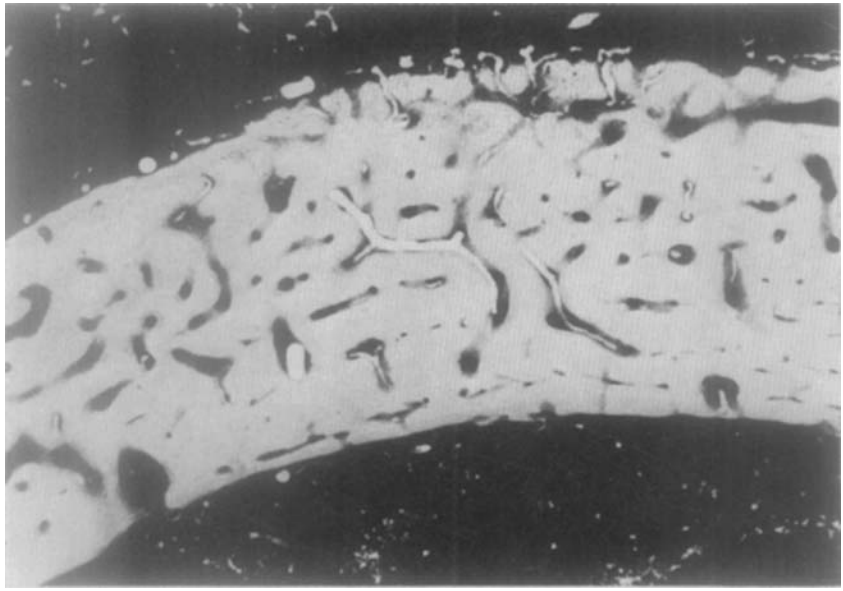


Fig. 44: 3 weeks, group 5 – L 6493 D (micro-angiogram x 40). Active and completed revascularization of the cortex, but as yet no normal features. The subperiosteal layer of new bone comprises a few small vessels perpendicular to the cortex. The medullary cavity contains new medullary blood vessels which are distinguishable from the radiopaque material of the modified acrylic cement.

In *groups 3 and 4* vessels are seen between acrylic cement and bone along the endosteal margin of the cortex. They have increased in number and size and sometimes form small „lakes". Otherwise the medullary cavity remains translucent.

In *group 5* the medullary vascularization increases further after 3 and after 6 months, and the same applies to the amount of bone tissue localized in the pores of the cement (fig. 47). After 6 months the intramedullary bone has matured further and is therefore better visible radiologically.

Observation period: 9, 12 and 24 months

In *group 3* the pattern of medullary vascularization shows no further essential changes.

4.6. *The normal fluorescence-microscopic features on the control side*

Knowledge of the normal process of growth remodelling of the metaphyses and diaphyses of long bones is essential in the understanding and

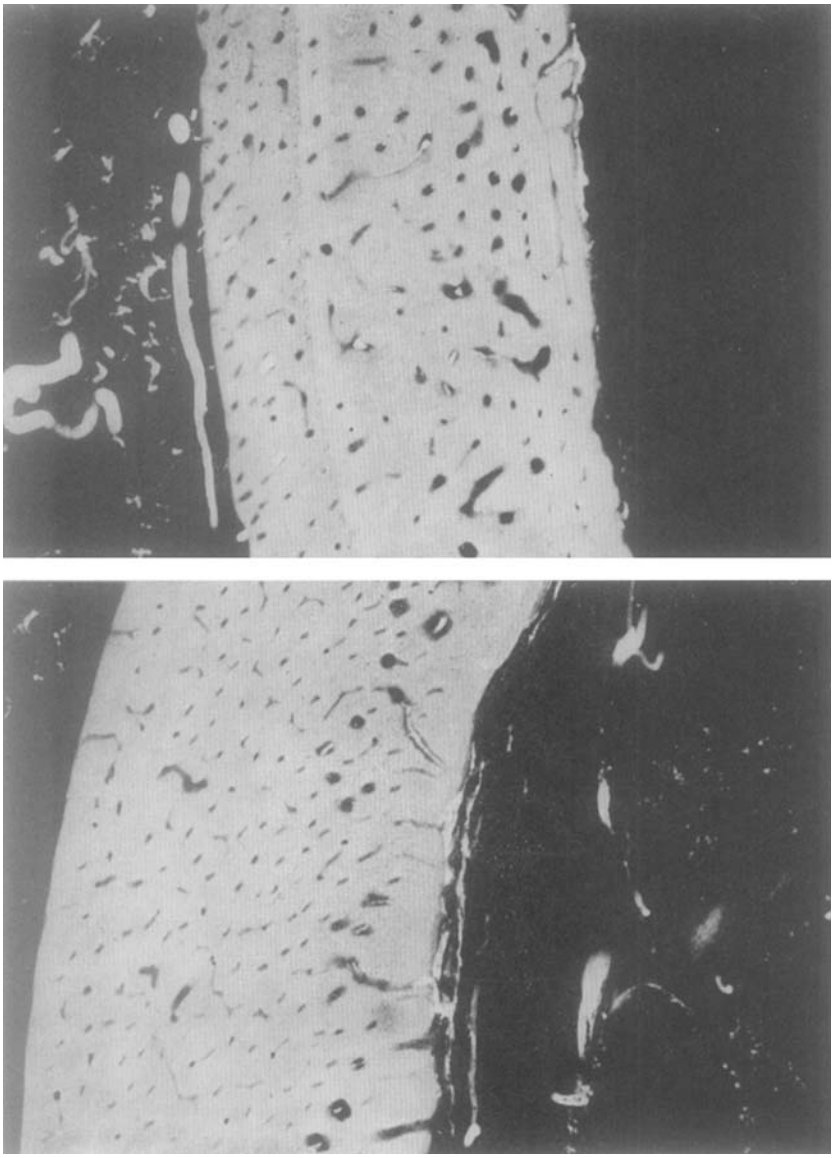


Fig. 45: 6 weeks, group 3 – L 6438 D - Su (micro-angiogram x 35). Between cement mass and bone lie new medullary vessels, which can form „lakes". The reactive vascularization in the subperiosteal bone is ebbing down, but there are still some sites with greatly increased extraosseous vascularization.

Fig. 46: 6 weeks, group 5 – L 6490 B (micro-angiogram x 40). There is marked regeneration of medullary blood vessels. The extraosseous circulation is normalized. Compare with fig. 45.

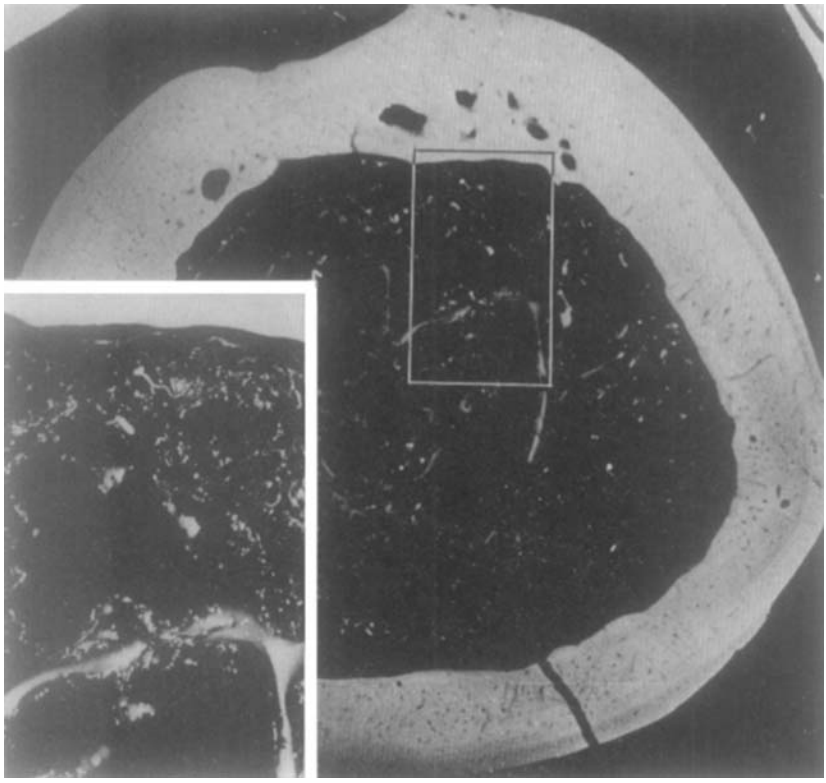


Fig. 47: 6 months, group 5 – L 6487 A (micro-angiogram x 10; detail x 24). General view to demonstrate the distribution of the bone spicules in the pores of the modified acrylic cement. The detail shows numerous new medullary vessels, also in the vicinity of the bone in the pores. Vessels viewed in cross-section are not readily distinguishable from the granules of contrast medium.

differentiation of the intraosseous regenerative processes reactive to the disturbance of medullary vascularization on introduction of acrylic cement into the medullary cavity on the one hand, and normal transverse growth on the other.

During longitudinal growth of a long bone the diameter of the diaphysis increases by periosteal apposition and endosteal resorption. As the bone increases in length, however, the metaphysis must decrease in diameter. Bone is removed from the outer surface of the metaphysis and endosteally deposited (fig. 48). This growth remodelling has been clearly demonstrated in autoradiographic studies of the rat tibia (Leblond et al. 1950) and the rabbit tibia (Owen et al. 1955). As the growing metaphysis moves in the direction of the bone end, previously metaphyseal areas are reloc-

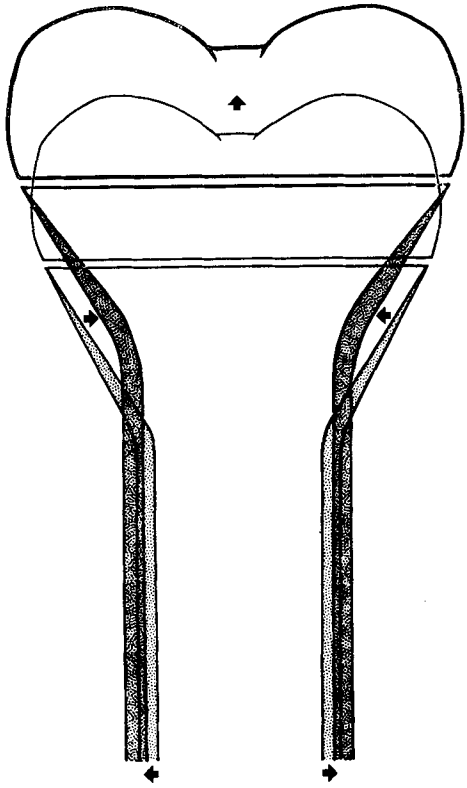


Fig. 48: Schematic representation of some aspects of growth remodelling of long bones. Arrows indicate the direction of growth.

ated in the form of the thinner diaphysis. The diaphysis assumes its definite typical size and dimensions during growth. Due to torsion and development of cristae, this apposition usually does not have the same width all around its diameter. In the metaphysis, too, the decrease in width is usually not circular due to the development of tuberosities, tubercles, ridges and grooves (Enlow 1968).

The above described growth remodelling patterns were also clearly demonstrated in the control femurs of the nearly 6-month-old rabbits by sequential labelling (fig. 49A).

4.7. *Fluorescence-microscopic findings after 7 weeks*

As has already been pointed out, in particular the animals observed by sequential fluorochrome labelling during 7 weeks contributed to the

completion of the picture obtained after studying the histological material. Since the changes on the endosteal side of the cortex were particularly important for this study, the expected differences between the 5 groups could be most clearly demonstrated in the distal, partly metaphyseal sections D.

Groups 1 and 2 show unmistakable restoration of the expected growth pattern in the diaphyseal as well as in the distal metaphyseal sections (fig. 49B). As compared with the control sections there is somewhat more bone remodelling, particularly in the inner cortical layers.

In *group 3* a very thin layer of bone proves to have been endosteally deposited at most sites. The inner half of the cortex shows hardly any osteogenesis, whereas the outer half is characterized by great activity. The new subperiosteal bone layer deposited during the first two post-operative weeks, is clearly demarcated from the old cortex (fig. 49C). A striking fact is that active remodelling processes are in progress throughout the diaphyseal as wel as the metaphyseal cortex in the region of the linea aspera (fig. 49F, G).

In *group 4* no endosteal bone apposition is observed either in the diaphyseal or in the metaphyseal sections. The subperiosteally deposited new bone layer attains a considerable thickness. Here, too, the bone remodelling processes in the region of the linea aspera are further advanced than anywhere else in the cortex, where activity can be observed only in the outer zone (fig. 49D).

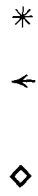
In all sections of *group 5* intramedullary bone – coloured red by Alizarin complexon – is found. Occasionally a green line (calcein green) is also observed. Endosteal bone apposition is demonstrable throughout the distal metaphyseal and the diaphyseal sections. Metaphyseally this bone is marked by several coloured lines (fig. 49E). Remodelling processes are in progress especially in the inner half of the cortex. New osteons are coloured by several fluorochromes.

4.8. *Macro-autoradiographic findings*

The autoradiographic control sections clearly demonstrated the growth remodelling of the longitudinally growing rabbit femur schematically shown in fig. 48 (figs. 50, 51, 52). The femurs were always paired for cutting so that on the film the treated femur could be compared with its control.

Observation period: 1 week

The autoradiograms of *groups 1 and 2* are identical. Both show increased periosteal activity, and an occasional dark line can also be observed in the metaphyseal endosteum (fig. 50).



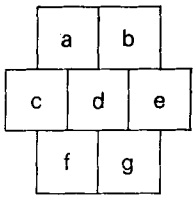


Fig. 49A: Normal fluorescence-microscopic features of the metaphyseal cortex of a rabbit aged nearly 6 months. R 6455 D (III RS position 3, x 36). New bone has been apposed during growth, particularly in the endosteum. Listed in the direction of the endosteum, the following fluorochromes were here deposited: terramycin, Alizarin complexon, xylenol orange, calcein green and Alizarin complexon. Calcein blue cannot be visualized with the optical system used.

Fig. 49B: 7 weeks, group 1 – R 6443 D (III RS position 3, x 36). An ample amount of bone has been endosteally apposed. Remodelling activity in the cortex slightly exceeds that in the control specimen. On the periosteal side, woven bone deposited in the first week is labelled with xylenol orange. The small tear is an artefact.

Fig. 49C: 7 weeks, group 3 – L 6448 D - Su (III RS position 3, x 36). The medullary cavity is translucent. A thin layer of new endosteal bone is labelled with Alizarin complexon. Considerable activity in the outer half of the cortex and in the subperiosteally apposed bone, which is separated from it by a terramycin line.

Fig. 49D: 7 weeks, group 4 – L 6447 D - Su–C (III RS position 3, x 36). No endosteal bone apposition. The subperiosteally apposed bone layer has the same thickness as the old cortex. Cortical bone remodelling is more advanced in the region of the linea aspera than elsewhere in the cortex.

Fig. 49E: 7 weeks, group 5 – L 6483 D (III RS position 3, x 36). Newly formed bone spicules coloured by Alizarin complexon (◇) are observed in the medullary cavity. A dead bone fragment has not been labelled by a fluorochrome (≺). A considerable amount of bone has been endosteally apposed (✳), and the cortex shows the vivid features of active remodelling. A thin layer of subperiosteal bone formed during the first postoperative week has been labelled by xylenol orange and Alizarin complexon.

Fig. 49F: 7 weeks, group 3 – L 6443 D - Pa (III RS position 3, x 11). Active remodelling is observed at the level of the linea aspera but much less in other parts of the cortex. *Fig. 49G* (x 17), enlargement from fig. 49F, to demonstrate the increased remodelling activity in detail.

Groups 3 and 4 do not differ either. There is greatly increased periosteal activity, and no labelling can be seen in the endosteum. Both animals don't even show any activity in the distal femoral epiphysis (fig. 51).

After introduction of modified acrylic cement (*group 5*), increased periosteal activity is likewise seen, while the endosteal bone has incorporated Strontium only at occasional sites in the metaphysis. The features resemble those observed in groups 1 and 2.

Observation period: 4 weeks

As compared with their controls, the femurs in *groups 1 and 2* still show slightly increased periosteal activity; endosteal activity has increased as compared with that in the controls.

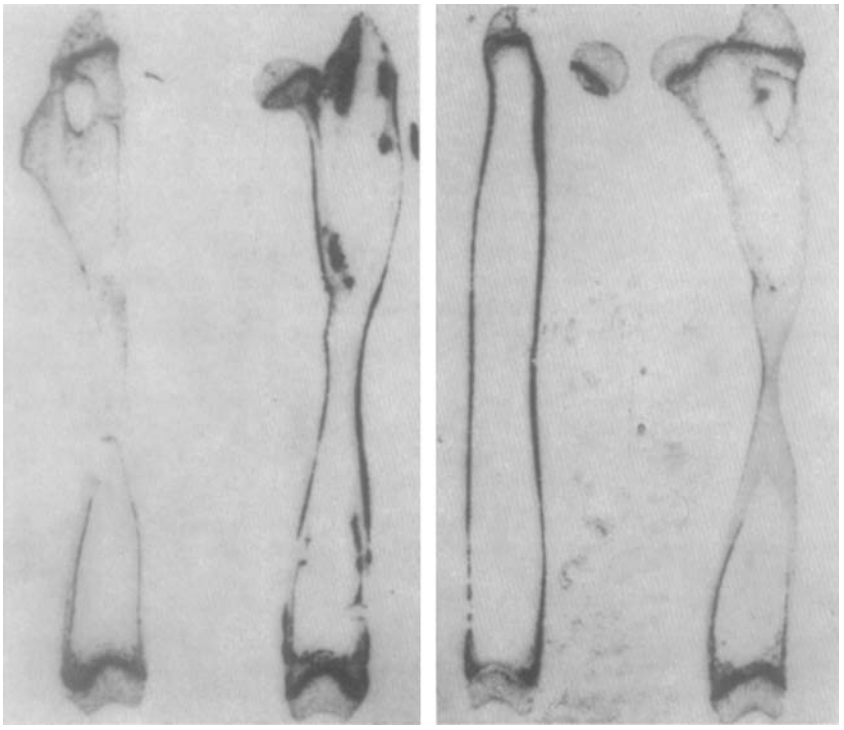


Fig. 50: 1 week, group 1 – 6463 (autoradiogram x 1). As compared with the control femur (left), periosteal activity has increased. Endosteal activity in the metaphysis has diminished.

Fig. 51: 1 week, group 4 – 6458 (autoradiogram x 1). Greatly increased activity on the bone surface (left); no endosteal labelling at all. The growth plate in the distal femur is „empty"! The right femur used as control.

In *group 3* the activity on the femoral surface is still slightly increased. Endosteal radioactive labelling is much weaker than that in the control bone, and is interrupted in several places. In *group 4* the femur shows as yet no endosteal activity at all. The distal femoral epiphysis is labelled in group 3 as well as in group 4.

In *group 5* the activity on the bone surface has virtually returned to normal, but endosteal labelling is weak and interrupted in several places.

Observation period: 4 months

The increased periosteal activity has returned to normal in *group 4*. Restored endosteal labelling is observed, although it is still interrupted at a few sites. No „blacking" has occurred in the medullary cavity.

As compared with the control bone, the treated femurs in *group 5*

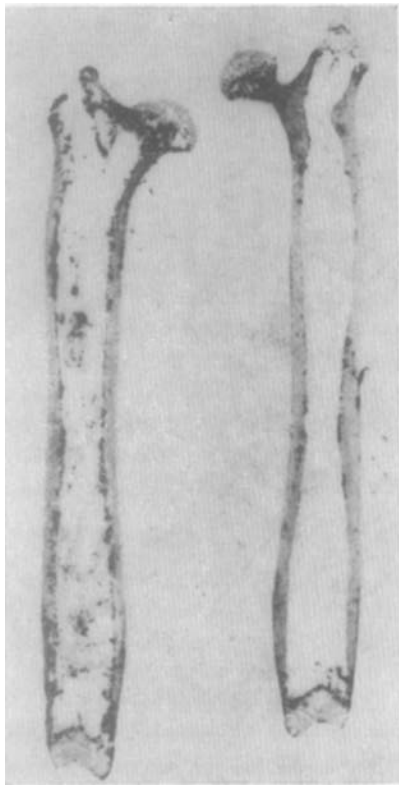


Fig. 52: 4 months, group 5 – 6563 (autoradiogram x 1). Normalized periosteal activity; slightly increased endosteal labelling. Unlike the medullary cavity of the control femur (right), which shows no „blacking", the bone grown into the cement pores has been labelled with Strontium^{89}!

show normalized periosteal but increased endosteal activity. In the medullary cavity, filled with modified acrylic cement, the bone grown into the pores is labelled with Strontium^{89} (fig. 52)!

4.9. *Discussion*

4.9.1. Discussion of results

The severity of the trauma inflicted could be deduced from the histological changes observed in medullary cavity, cortex and periosteum after disturbance of the medullary vascularization and subsequent introduction of acrylic cement. Although in this experiment there seemed to be an evident correlation between the amounts of newly formed subperiosteal

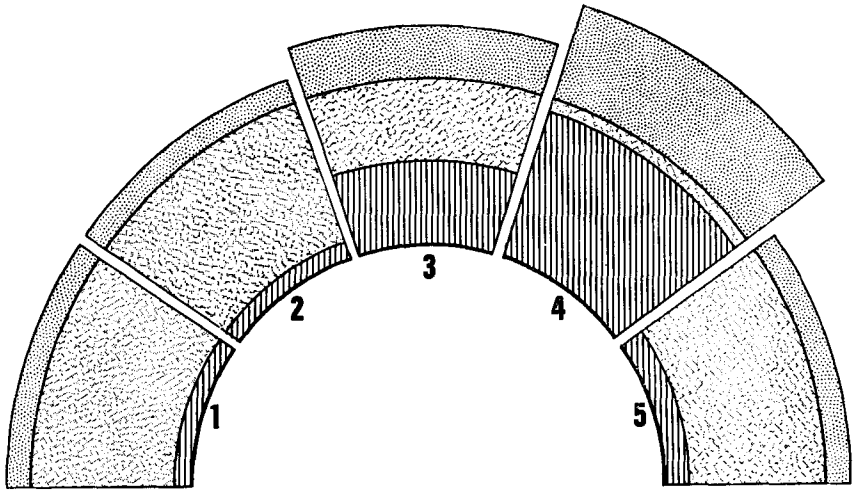


Fig. 53: Schematic representation of the extent of the cortical necrosis (shaded area) and the reactive development of subperiosteal new bone (dotted area) in the 5 groups 2 weeks after operation.

bone and the extent of the cortical necrosis, yet the cortical necrosis was the principal yardstick used in evaluation of results. The periosteum of immature rabbits (used in this study; fig. 7) is known to be more richly vascularized than that in adult animals. After reaming of the medullary cavity of the rabbit tibia, Danckwardt-Lilliestrom (1969) found that growing animals were forming a substantially larger amount of new subperiosteal bone than adult animals. The same was observed in the experiments of Trueta and Cavadias (1955) with Küntscher nailing of rabbit radii. Intensity and extent of the cortical necrosis could definitely be determined in the histological material after 2 weeks (fig. 53). The cortical necrosis was to be regarded as the result of a single event (Willert and Puls 1972), and could be directly related to the trauma inflicted, the destruction of the medullary blood vessels, and the type of cement introduced.

In *group 1* the enormous regenerative power of the medullary vascular system was confirmed after complete destruction of the medullary blood vessels by reaming of the medullary cavity and suction drainage of medullary contents (fig. 38A; fig. 40). By virtue of a vent cut in the femur, which prevented spread of the cortical ischaemia as a result of intracortical circulation block caused by fat embolism (Danckwardt-Lilliestrom 1969; Danckwardt-Lilliestrom et al. 1970b), the cortical necrosis was confined to a narrow zone on the endosteal side (fig. 53). From the

second postoperative week on, a new endosteal layer of bone was already deposited from a regenerated endosteum (fig. 13; fig. 50); in terms of thickness, this layer was no longer distinguishable from the normal growth remodelling in the control sections after 6 weeks (fig. 18) and 7 weeks (fig. 49B). After 3 months the necrotic tissue was found to have been almost completely replaced by vital bone (fig. 24), and after 6 months restoration could be described as complete.

No essential histological (figs. 14, 19, 25) and (micro-)angiographic differences (figs. 38C, 41) between group 1 and *group 2* were demonstrable (fig. 53). Apparently the residual monomer, gradually diffusing from the set material, had had no detrimental effect. This was confirmed by the connective tissue membrane which developed around the Palacos rod from the 2nd week on. The set-up of this experiment thus made it possible to corroborate the view of the authors quoted by Contzen et al. (1967), Slooff (1970) and Charnley (1970) that polymerized acrylic cement which is not submitted to stress is a biocompatible material (cf. chapter 1, page 25).

In *group 3*, necrosis of the inner one-half to two-thirds of the cortex was observed 2 weeks after complete destruction of the medullary vascularization and additional introduction of the commercial cements Palacos and Sulfix-6 (fig. 15). The extent of this necrosis, schematically represented in fig. 53, can only have been caused by local side effects of acrylic cement – high polymerization temperature and cytotoxic monomer – if compared with the findings in group 1. No essential differences were histologically demonstrable between the commercial acrylic cements Palacos and Sulfix-6 applied in the two series (groups 3A and 3B).

Organization of this necrosis was very slow, and associated with the development of large resorption lacunae (fig. 20) which were sometimes filled with bone marrow (figs. 26, 31), and sequestration of necrotic bone into the medullary cavity (figs. 34, 36). From the 4th week on, a new endosteum was observed from which a very modest endosteal bone layer was deposited (figs. 20, 49C, 49F, 49G). In comparable experiments carried out on dogs by Slooff (1970, 1971) and Lindwer (1972, 1975), incipient formation of a connective tissue membrane was already observed after 2 weeks; in our experiment this did not appear until later. A first indication was observed after 7 weeks, and only after 6 months was a connective tissue membrane a constant finding. Since the periosteum in dogs reacts by more extensive formation of new bone (Danckwardt-Lilliestrom 1969; Slooff 1970, 1971), it is possible that this difference should also be ascribed to the animal species used.

Animal experiments of the type reported by Rietz (1968) and Szyszkowitz (1971), in which artificial lesions of bone were repaired by intramedullary and interfragmental introduction of acrylic cement (Szyszkowitz 1971), or by intramedullary

prostheses anchored with cement (Rietz 1968), are in our opinion hardly conclusive in assessing the tissue compatibility or incompatibility of acrylic cement. The disturbance of the intramedullary vascularization is so extensive (and so unfavourably influenced by the instability of the implants) that it seems at best possible to conclude that this is an unsuitable method of osteosynthesis and an unsuitable experimental set-up for assessment of the tissue compatibility of acrylic cement.

Restitutio ad integrum was not ultimately achieved. After 24 months the entire inner half of the cortex proved to have been replaced by vital bone marrow. This in turn was separated from the cement by a thick connective tissue membrane (fig. 37). This finding was in agreement with the observations of Lindwer (1972, 1975), who introduced commercial acrylic cement into dog femurs after removing medullary contents. In similar experiments on dogs, Slooff (1970, 1971) observed identical short-term histological changes (maximum observation period 109 days). This very slow and incomplete repair of the tissue damage inflicted will have to be explained largely on the basis of markedly delayed regeneration of the medullary vessels (figs. 42, 45). Revascularization appears to be greatly impeded when a solid mass of cement blocks completely the medullary blood flow, unlike the medullary revascularization which can occur in the flutes of a tight-fitting nail (Rhinelander 1972, 1973). Corroborative evidence is supplied by the observations of Brookes and Gallannaugh (1975), who in a haemodynamic study following implantation of acrylic cement in rat tibiae established hypovascularity of the bone after as many as 16 weeks. Unlike Lindwer, who from his experiments derived far-reaching conclusions and recommendations for the clinical use of acrylic cement, we would only conclude that acrylic cement setting in situ can be described as a biologically non-inert substance which can cause permanent structural changes in bone tissue.

The most severe tissue damage was found in *group 4*. After interruption of the medullary vascularization and introduction of an acrylic „cement" with an abundance of monomer (about 30 volume per cent), virtually complete necrosis of the cortex was observed after 2 weeks (fig. 53). This necrosis was not only very extensive but also very intensive (fig. 16). Histological and autoradiographic (fig. 51) examination disclosed enormous periosteal activity, the cortical diameter even being doubled at some sites (fig. 49D). The regeneration of the cortex was even slower than that in group 3 (figs. 21, 27). After 7 weeks new endosteal bone had still not been formed (fig. 49D). Only after 3 months (fig. 27) was this histologically demonstrable, and the autoradiogram after 4 months showed only scanty endosteal activity. After 6 months there was as yet no histological evidence that the process of cortical regeneration was completed (fig. 32). As in group 3, the incomplete and delayed revascularization of the medullary cavity must have been an important factor in this respect (fig. 43). Since the „cement" did not polymerize and therefore produced no heat,

the tissue damage (in so far as it was not caused by the interruption of the medullary vascularization) can be ascribed to the cytotoxic monomer. It is quite conceivable that very intensive necrosis could result from invasion of the bone by monomer leaking through the cortical canals.

The effect of an overdose of monomer becomes even more apparent when we compare the above findings with those in *group 5*, in which the high temperature factor was likewise excluded. After complete destruction of the medullary vascularization (fig. 38B) and additional intramedullary application of a modified acrylic cement, observations after 2 weeks showed a slightly more extensive cortical necrosis than that in groups 1 and 2. This is schematically represented in fig. 53.

Regeneration of the necrotic part of the cortex (fig. 17) was significantly more rapid than that in groups 3 and 4 (figs. 22, 28). The histological findings closely resembled those obtained in the first two groups. Particularly characteristic in this respect were the fluorescence-microscopic features of the endosteal cortex apposition after 7 weeks (fig. 49E). New, well-vascularized bone and bone marrow tissue proved to grow into the pores of the gel-cement from the 6th week onwards (figs. 23, 49E). The longer the observation period, the heavier was the growth of bone into the cement pores (figs. 29, 30, 35, 52). This was a constant finding.

There was even almost complete restoration of the cortex after 6 months (fig. 33), so that the situation after application of the modified acrylic cement could be described as one of transient necrosis with maintenance of the integrity of the cortical structure. Undoubtedly, this favourable course must have been made possible by the rapid, intensive regeneration of medullary vessels which was observed after as few as 2 weeks in histological sections as well as in micro-angiograms (figs. 44, 46, 47). This early and vigorous revascularization of the medullary system contrasted sharply with the slow and sparse restoration of the medullary circulation in groups 3 and 4.

Applying the „subtraction" method mentioned in chapter 3 (page 48) to groups 3 and 5, we are left with the high polymerization temperature of the commercial acrylic cement, since the monomer concentration in Palacos, Sulfix-6 and modified Sulfix-6 remained the same. The significant difference in extent of cortical necrosis between the two groups will therefore have to be explained on the basis of the temperature effect of acrylic cement setting in situ. The principal local untoward side effect of acrylic cement is therefore the high polymerization temperature. Of course this does not exclude any possible local side effect of the cytotoxic monomer. The disastrous effect of an overdose of monomer was clearly demonstrated in group 4. Apparently small amounts of monomer produce no tissue damage (group 2). In order to quantify the monomer effect, a comparison could be made between group 5 and group 1, the difference being described as monomer effect. In this comparison, however, the

effect of the filling of the medullary cavity is introduced as an unknown factor. The results of this experiment, therefore, warrant no exact conclusion as to the quantitative cytotoxic effect of the monomer escaping during polymerization. It seems justifiable, nevertheless, to describe this effect as minimal when compared with the effect of the high polymerization temperature.

4.9.2. Discussion of the techniques used

The micro-angiographic technique used made it possible to fill the vessels on the afferent side of the circulation in so far as they were functional at the time of Micropaque perfusion. The technique failed to visualize a sufficient vascular area to warrant any conclusion about the direction of flow. Rhinelander's concept (fig. 3) of the circulation in diaphyseal cortical bone could therefore be neither confirmed nor refuted. Essential differences between Rhinelander's technique (Rhinelander et al. 1962) and ours were to be found in the perfusion technique, thickness and calcium content of the section, and the use of a stereo-viewer (Rhinelander uses decalcified sections, 1 mm thick, which are radiographed from two different angles and viewed with a stereo-viewer). Because our sections were used for fluorescence microscopy after microradiography, they had to be 100 micra thick and could not be decalcified.

Some support for Rhinelander's theory could be found in the fluorescence-microscopic findings in groups 3 and 4. It was evident that unmistakably more new osteons had been formed in the region of the lineae asperae* than elsewhere in the cortex (figs. 49C, D, F, G). This might be explained by the fact that the outer one-third of the cortex is supplied by periosteal arterioles in areas of heavy fascial attachment. Our micro-angiographic technique was insufficient to confirm this.

The fluorescence technique used was satisfactory. The new fluorochromes calcein blue and green, xylenol orange and Alizarin complexon produced distinct differences in colour and were virtually free from fading. The calcein green dose used proved to be too large because this fluorochrome superseded the other colours; this posed some problems in photography. The colour intensity of Alizarin Red S was not very satisfactory.

Little new information was obtained from the macro-autoradiograms.

---

* Unlike the dog femur, the rabbit femur has two lineae asperae. The posterolateral line extends from the third trochanter to the lateral supracondylar tuberosity; the posteromedial line extends from the lesser trochanter to the medial supracondylar tuberosity. Barone et al. (1973) referred to a labium laterale and a labium mediale, with the facies aspera in between.

Micro-autoradiography would be preferable for further investigations. Finally, the dissolution of the acrylic cement during the processing of the material was experienced as a disadvantage.

4.10. *Conclusions*

1. Complete interruption of the medullary circulation by reaming and suction of the rabbit femur provokes a number of typical reactions in bone marrow, cortex and periosteum. Ischaemic necrosis develops on the endosteal side of the cortex. This necrosis increases after additional application of acrylic cement in the medullary cavity.
2. Commercial acrylic cement setting in situ is a biologically non-inert material which causes local tissue damage.
3. The principle side effect responsible for this damage is the high polymerization temperature. The thermic tissue damage caused by acrylic cement far exceeds the necrosis which results from ischaemia.
4. An overdose of monomer (about 30 volume per cent) causes severe tissue damage. Minute amounts of monomer diffusing from set acrylic cement (so-called residual monomer) are not detrimental. The monomer which escapes during polymerization after implantation of acrylic cement in a dough-like state, may also contribute slightly to the resulting tissue damage.
5. A new modified acrylic cement which eliminates the high polymerization temperature is found to be much more readily tolerated by the tissues than the commercial acrylic cements Palacos and Sulfix-6. These do not differ essentially in terms of tissue damage caused. In the porous modified cement, bone is observed to grow into the pores from the 6th postoperative week on. This in-growth of bone runs parallel to the period of observation.

CHAPTER 5

CLOSING REMARKS

Many factors contribute to the success of an implant arthroplasty. The best results are obtained by careful determination of indications, an optimal surgical technique, adequate cementing, the use of a prosthesis of high mechanical properties, prevention of infection and adequate post-operative guidance of the patient. The initial fixation of the prosthesis has been greatly simplified by the introduction of acrylic cement. The question is how long the good result lasts. In the long run a number of new problems present themselves. Late loosening of the prosthesis and inexplicable complaints of pain necessitate more often revision operations. The principal problem of implant arthroplasty continues to be the bone-implant interface. It is in this interface that biologically and biomechanically undesirable tissue changes occur in reaction to acrylic cement (Willert and Puls 1972; Walker 1973; Müller 1974a, 1974b; Hirsch 1974).

This study has clearly demonstrated that the high polymerization temperature, even with the very small quantities used in this study, is the principal untoward side effect of acrylic cement. It has been explained in chapter 1 (section 1.2.3.) that there is no agreement how much the temperature rises in the interface between bone and acrylic cement. In the German literature a temperature of 56° C is accepted as critical limit.

Burns caused by acrylic cement have been described in a few case reports (Jefferis 1971). The remnants of the acrylic cement used in a total hip arthroplasty burned through the surgical drapes and caused lesions of the patient's abdominal skin. The threshold value for thermic necrosis of epithelial cells is 52-55° C at an exposure time of 30 seconds (Kuhl et al. 1954; Moritz and Henriques 1947, quoted by Lundskog 1972).

The superbly documented experiments on the immediate effect of various temperatures and exposure times on vital bone tissue, performed by Lundskog (1972) showed conclusively that the osteocytes have about the same threshold value for thermic necrosis as epithelial cells in cutaneous tissue. At an exposure time of 30 seconds, the threshold value for osteocytic necrosis was found to be 50° C (Lundskog 1972). Moreover, a

direct correlation was demonstrated between the extent of cellular necrosis on the one hand, and exposure time and temperature on the other (Matthews and Hirsch 1972; Lundskog 1972). The hypothesis (pages 17, 18) that thermic necrosis would be less in the case of an intact blood circulation, was not confirmed. Both Matthews and Hirsch (1972) and Lundskog (1972) considered it unlikely that the cooling effect of the cortical circulation could be important in this respect. The high temperature to which the bone is exposed after implantation of acrylic cement, persists several minutes (fig. 1, 5). Our own conclusion therefore is that, while a temperature of 56° C may be the critical limit for denaturation of certain proteins (Lehnartz 1959), the limit for cellular bone necrosis caused by acrylic cement is significantly lower.

The imperfections of traditional implant materials have prompted further investigation. Granted that the ultimate purpose is the achievement of a biological fixation between implant and bone, several porous materials have recently been developed, which combine adequate tissue compatibility with sufficient material strength. Investigators of biomaterials have had to do an enormous amount of work before the most suitable materials could be selected and the suitable porosity percentage and minimal pore size, shape and interconnection permitting in-growth of hard tissues could be determined. The substantial body of information available on these implant materials has been recently compiled by such authors as Homsy et al. (1972), Hulbert et al. (1973) and Smith (1974). Bone in-growth has been demonstrated in porous ceramics, carbons, metals and polymers. With the aid of certain types of glass (bioglass) an intensive chemical binding with bone can be achieved (Hench and Paschall 1974).

However, these porous materials do not ensure immediate fixation in bone, as does acrylic cement. For orthopaedic applications, prostheses made of porous material or given a porous coating would have to be exactly made to measure in advance to ensure intimate contact with the implant bed. Next, movements between bone and implant would have to be prevented for a number of months, or a temporary fixation would have to be used until sufficient bone in-growth had taken place. For temporary fixation the use of biostable or partly biodegradable bone cements has been suggested (Griss et al. 1973).

The modified acrylic cement used in our experiments comes close to this ideal. The aqueous gel phase is biodegradable. Good tissue acceptance is achieved by drastic reduction of the high temperature effect.

In two other new cements used in dentistry, the high temperature effect has also been eliminated (PAZ) or slightly reduced (MMA-TBB). Zinc polyacrylate (PAZ) consists of an aqueous solution of polyacrylic acid and zinc oxide (Smith 1968). MMA-TBB is a cement based on polymethacrylate, with tributylborane as special catalyst system. Both materials are described as capable of chemical binding to

organic components of hard tissues. For orthopaedic applications these cements are still in the experimental stage (Peters et al. 1972; Iida et al. 1974), but they should be mentioned here as possible alternatives for acrylic cement.

The decrease in temperature of the modified cement results on the one hand from the good heat-conducting capacity of the aqueous phase, and on the other hand from a relative reduction of the amount of monomer by admixture of the gel (de Wijn 1975). A possible disadvantage of the lower maximum temperature lies in a probably less complete polymerization of the mixture, in view of which some loss of mechanical properties is to be expected (Puhl and Schulitz 1971). However, an attempt to alter the maximum temperature can be made only at the cost of some decrease in mechanical strength. An advantage of the lower temperature is that less shrinkage occurs as a result of lower thermic cooling. The smaller percentage of acrylic cement also contributes to this. In view of the hydrophilic properties of the gel, the cement can in fact be expected to expand.

Other interesting features are that the duration of the curing time does not differ from that of the commercial product (fig. 5), and that substances such as antibiotics, cytostatics, etc. can be dissolved in the gel (de Wijn 1975).

At 35 per cent porosity, a pore diameter of 50-150 micra and good pore interconnection – all readily reproducible characteristics – the material is fairly strong; when compared with commercial cement its compressive strength is about 50 per cent (de Wijn 1975). In this loss of mechanical strength it does not differ from other porous materials. However, in-growth of hard tissues (figs. 23, 29, 30, 35, 46, 47, 49E, 52) can considerably reinforce the material in situ and (more important) effect optimal fixation between bone and implant. The mechanisms of bone in-growth in porous materials are still unexplained (Hulbert et al. 1973). Strong arguments have been presented in support of the suggestion that electrical phenomena are directly involved in osteogenesis (Bassett 1971). Applying the three criteria for bone induction formulated by Chalmers et al. (1975), it could be postulated that: 1) the porous polymer is the *inducing stimulus*; it works as a dipole, producing free surface energy (Bassett 1971, 1975); 2) young bone marrow cells act as *osteogenic precursor cells*; 3) the CMC gel creates a *favourable environment for osteogenesis.*

Further research with this new biocompatible material may lead to improvements. It is important to establish whether the modified acrylic cement is sufficiently strong for temporary fixation of a prosthesis, and whether under stress (Bassett 1971; Hulbert et al. 1974) bone in-growth can be accelerated and increased to such an extent that biological fixation between bone and implant results. Another possible application might be that of filling substance for cancellous or non-weightbearing bone defects.

Discussing the physical properties of commercial acrylic cement, Lautenschlager et al. philosophized in 1974: „Perhaps someday superior cements will be developed. Cements where such things as exothermic temperature rise and residual monomer from incomplete polymerization are not potential problems. Perhaps someday cements of a controlled surface porosity for tissue in-growth to aid fixation, cements with mechanical properties matched to bone and prosthesis alike to forever forbid loosening, and cements with additives which will produce proper radiopacity and contribute to the prevention of infection will become available".

Some of the ideal properties enumerated in this statement seem to have been incorporated in this material. The future will show whether it is clinically applicable.

SUMMARY

The three factors generally regarded as causes of the tissue changes in bone after acrylic cement implantation are the high polymerization temperature of acrylic cement, the cytotoxicity of the monomer and the interruption of the osseous vascularization by the surgical preparations for the implantation. The principal objective of this experimental study was to establish which of these three factors is the most important. The rabbit femur was chosen as experimental model.

Chapter 1 presents recent views on the physical, chemical, pharmacological and biological properties of acrylic cement when applied as filler substance for the fixation of prostheses in bone. The tissue changes which occur in the new joint capsule and the bony implant bed after total hip replacement are described.

Chapter 2 discusses the topographical anatomy of the vascularization of the rabbit femur and the functional anatomy of the cortical vascularization of the diaphysis of long bones in general. Intramedullary interventions which result in complete block of the medullary circulation, disturb the physiological pattern of vascularization. The changes in the diaphyseal cortical circulation and the reactions provoked by ischaemia in medullary cavity, cortex and periosteum are discussed. In this context great importance is attached to the increased intramedullary pressure and the intracortical bone marrow embolism related to it.

Chapter 3 explains the argumentation underlying the experiments. Three types of cement are used: 1) commercial acrylic cement; 2) a catalyst-free „acrylic cement" in which no polymerization reaction (and therefore no heat generation) occurs, an excess of monomer being left; 3) a modified porous acrylic cement in which the high temperature effect has been drastically reduced, the monomer content remaining unchanged. The operative technique used is described and discussed. The grouping of the test animals in accordance with the cement type implanted is explained. The chapter closes with a discussion of the laboratory techniques used.

Chapter 4 presents an analysis of the results obtained, with emphasis on a comparison of the tissue changes observed in the different groups. The findings obtained in the histological material are supplemented with (micro)angiographic, fluorescence-microscopic and autoradiographic re-

sults. After discussion of the results and of the techniques used, the chapter closes with a number of conclusions.

The results obtained in the experiments provide a definite answer to the question posed in the problem statement. After implantation, the high polymerization temperature of the acrylic cement causes changes in bone tissue which in intensity and extent far exceed the ischaemic cortical necrosis due to the surgical preparation of the medullary cavity (reaming and suction). Whether the monomer escaping after implantation of acrylic cement contributes to the tissue changes is not certain. If it does, then its contribution is certainly a small one.

A modified porous acrylic cement (Sulfix-6 with CMC gel), which differs from commercial acrylic cements in that the high polymerization temperature as tissue-damaging factor has been eliminated, shows unmistakably superior tissue tolerance. Moreover, shortly after its implantation growth of well-vascularized bone into the pores of the unstressed cement is observed; a growth which increases with the period of observation.

Chapter 5 discusses, among other things, the potential clinical application of this new porous acrylic cement.

REFERENCES

Adkins, K. F. (1965). Alisarin Red S as an intravital fluorochrome in mineralizing tissues. *Stain Technol.* 40, *69-70.*

Amstutz, H. C. (1970). Complications of total hip replacement. *Clin. Orthop.* 72, *123-137.*

Anderson, L. D., W. R. Hamsa, Th. L. Waring (1964). Femoral-head prosthesis: A review of 356 operations and their results. *J. Bone Jt. Surg.* 46-A, *1049-1065.*

Anderson, L. D. (1965). Compression plate fixation and the effect of different types of internal fixation on fracture healing. *J. Bone Jt. Surg.* 47-A, *191-208.*

Andersson, G. B. J., M. A. R. Freeman, S. A. V. Swanson (1972). Loosening of the cemented acetabular cup in total hip replacement. *J. Bone Jt. Surg.* 54-B, *590-599.*

Barone, R., C. Pavaux, P. C. Blin, P. Cuq (1973). *Atlas d'anatomie du lapin. Atlas of Rabbit Anatomy.* Masson, Paris.

Bassett, C. A. L. (1971). Biophysical principles affecting bone structure. In: *The biochemistry and physiology of bone, 2nd ed. (G. H. Bourne ed.), vol. III, pp. 1-69.* Academic Press, New York.

Bassett, C. A. L. (1975). Personal communication.

Beneke, G., R. Kuprasch, W. Mohr, K. Paulini and W. Mohing (1973). Die Reaktion der Gelenkkapsel nach Totalarthroplastik des Hüftgelenkes. *Arch. Orthop. Unfall-Chir. 75, 289-301.*

Berman, A. T., H. L. Price and J. F. Hahn (1974). The cardiovascular effects of methylmethacrylate in dogs. *Clin. Orthop.* 100, *265-269.*

Biehl, G., J. Harms and U. Hanser (1974). Experimentelle Untersuchungen über die Wärmeentwicklung im Knochen bei der Polymerisation von Knochenzement. Intraoperative Temperaturmessungen bei normaler Blutzirkulation und in Blutleere. *Arch. orthop. Unfall-Chir.* 78, *62-69.*

Blair Fries, I., A. A. Fisher and E. A. Salvati (1974). The allergenic effects of methyl-methacrylate bone cement. *J. Bone Jt. Surg.* 56-A, *1308.*

Bloch, B., J. K. Haken and G. W. Hastings (1970). Evaluation of cold-curing acrylic cement for prosthesis stabilization. *Clin. Orthop.* 72, *239-241.*

Bloch, B. and G. W. Hastings (1972). *Plastics materials in surgery, 2nd ed.* Thomas, Springfield, U.S.A.

Bloch, B. (1974). *Immunological problems after total hip replacement.* Paper read at the combined meeting of the orthopaedic departments of Leuven and Nijmegen Universities, November 16th, 1974, Nijmegen.

Böhler, L. and J. Böhler (1949). Küntscher's medullary nailing. *J. Bone Jt. Surg.* 31-A, *295-305.*

Bragdon, J. H., L. Foster and M. C. Sosman (1949). Experimental infarction of bone and marrow. *Am. J. Path.* 25, *709-723.*

Brånemark, P-I. (1959). Vital microscopy of bone marrow in rabbit. *Scand. J. Clin. Lab. Invest.* 11, *Suppl. 38.*

Brånemark, P-I., U. Breine, B. Johansson, P. J. Roylance, H. Röckert and J. M. Yoffey (1964). Regeneration of bone marrow. A clinical and experimental study following removal of bone marrow by curettage. *Acta Anat.* 59, *1-46.*

Brinkmann, K. E. and K. Heilmann (1974). Klinische, röntgenologische und feingewebliche Untersuchungen an ausgelockerten Hüftgelenksprothesen. *Arch. orthop. Unfall-Chir.* 80, *333-342.*

Brittain, G. J. C. and D. J. Ryan (1972). Hypotension and methylmethacrylate cement. *Brit. Med. J.* 4, *667-668.*

Brookes, M. and R. G. Harrison (1957). The vascularization of the rabbit femur and tibiofibula. *J. Anat.* 91, *61-72.*

Brookes, M. (1958). The vascularization of long bones in the human foetus. *J. Anat.* 92, *261-267.*

Brookes, M. (1960). Sequelae of experimental partial ischaemia in long bones of the rabbit. *J. Anat.* 94, *552-561.*

Brookes, M. (1964). The blood supply of bone. In: *Modern trends in orthopaedics - 4: Science of fractures. (J. M. P. Clark, ed.), pp. 91-125,* Butterworth & Co., London.

Brookes, M. (1971). *The blood supply of bone.* Butterworth & Co., London.

Brookes, M. and S. C. Gallannaugh (1975). Circulatory depression in bone after acrylic implantation. *Clin. Orthop.* 107, *274-276.*

Buchholz, H. W. and H.-D. Gartmann (1972). Infektionsprophylaxe und operative Behandlung der schleichenden tiefen Infektion bei der totalen Endoprothese. *Chirurg* 43, *446-453.*

Buchholz, H. W. (1973). Die tiefe Infektion bei der totale Endoprothese. In: *Der totale Hüftgelenkersatz (H. Cotta and K.-P. Schulitz ed.), pp. 153-159.* G. Thieme Verlag, Stuttgart.

Cavadias, A. X. and J. Trueta (1965). The vascular contribution to the callus of fracture. *Surg. Gynec. Obstet.* 120, *731-747.*

Chalmers, J., D. H. Gray and J. Rush (1975). Observations on the induction of bone in soft tissues. *J. Bone Jt. Surg.* 57-B, *36-45.*

Charnley, J. (1960). Anchorage of the femoral head prosthesis to the shaft of the femur. *J. Bone Jt. Surg.* 42-B, *28-30.*

Charnley, J. (1961). Arthroplasty of the hip. A new operation. *The Lancet* I, *1129-1132.*

Charnley, J. (1965). A biomechanical analysis of the use of cement to anchor the femoral head prosthesis. *J. Bone Jt. Surg.* 47-B, *354-363.*

Charnley, J. (1970). *Acrylic cement in orthopaedic surgery.* Churchill Livingstone, Edinburgh-London.

Charosky, C. B., P. G. Bullough and P. D. Wilson (1973). Total hip replacement failures. A histological evaluation. *J. Bone Jt. Surg.* 55-A, *49-58.*

Chen, S. C., S. A. Lowe, J. T. Scales and R. H. Ansell (1974). An in vitro experiment to determine the efficiency of fixation of the McKee-Farrar acetabular component in relation to torsional force. *Acta Orthop. Scand.* 45, *429-435.*

Contzen, H., F. Straumann and E. Paschke (1967). *Grundlagen der Alloplastik mit Metallen und Kunststoffen.* G. Thieme Verlag, Stuttgart.

Convery, F. R., D. R. Gunn, J. D. Hughes and W. E. Martin (1975). The relative safety of polymethylmethacrylate. A controlled clinical study of randomly selected patients treated with Charnley and Ring total hip replacements. *J. Bone Jt. Surg.* 57-A, *57-64.*

Cotta, H. and K.-P. Schulitz (1970). Komplikationen der Hüft alloarthroplastik durch periartikuläre Gewebereaktionen. *Arch. orthop. Unfall-Chir.* 69, *39-59.*

Crock, H. V. (1967). *The blood supply of the lower limb bones in man.* E. & S. Livingstone ltd, London.

Danckwardt-Lilliestrom, G. (1969). Reaming of the medullary cavity and its effect in diaphyseal bone. A fluorochromic, microangiographic and histologic study on the rabbit tibia and dog femur. *Acta Orthop. Scand.* Suppl. 128.

Danckwardt-Lilliestrom, G., G. L. Lorenzi and S. Olerud (1970a). Intramedullary nailing after reaming. An investigation on the healing process in osteomized rabbit tibias. *Acta Orthop. Scand.* Suppl. 134.

Danckwardt-Lilliestrom, G., G. L. Lorenzi and S. Olerud (1970b). Intracortical circulation after intramedullary reaming with reduction of pressure in the medullary cavity. A microangiographic study on the rabbit tibia. *J. Bone Jt. Surg.* 52-A, *1390-1394.*

Dandy, D. J. (1974). The safety of acrylic bone cement. *Injury,* 5, *169-174.*

Dandy, D. J. and B. C. Theodorou (1975). The management of local complications of total hip replacement by the McKee-Farrar technique. *J. Bone Jt. Surg.* 57-B, *30-35.*

Debrunner, H. U. (1974). Die Erwärmung von Knochenzement bei der Polymerisation. *Arch. orthop. Unfall-Chir.* 78, *309-318.*

Debrunner, H. U. (1975). Die Volumenänderungen von Knochenzementen während der Härtung. *Arch. orthop. Unfall-Chir.* 81, *37-44.*

Debrunner, H. U. and A. Wettstein (1975a). Die Verarbeitungszeit von Knochenzementen. *Arch. orthop. Unfall-Chir.* (in press).

Debrunner, H. U. and A. Wettstein (1975b). Gewichtsverluste durch Verdunsten bei der Polymerisation von Knochenzement. Vorlaufige Mitteilung. Technischer Rapport 10/74. Abteilung für Experimentelle Orthopädie, Inzelspital, Bern.

Deutman, R. (1974). *Experience with the McKee-Farrar total hip arthroplasty.* Thesis, Leiden, Netherlands.

Dustmann, H. O., K.-P. Schulitz and H. Koch (1972). Fettembolie nach Anwendung von Knochenzement bei Hüftgelenkersatz. Tierexperimentelle Untersuchung. *Arch. orthop. Unfall-Chir.* 72, *114-121.*

Ellis, R. H. and J. Mulvein (1974). The cardiovascular effects of methylmethacrylate. *J. Bone Jt. Surg.* 56-B, *59-61.*

Enlow, D. H. (1968). *The human face. An account of the postnatal growth and development of the craniofacial skeleton, pp. 1-101.* Hoeber Medical Division, Harper & Row, New York.

Evans, E. M., M. A. R. Freeman, A. J. Miller and B. Vernon-Roberts (1974). Metal sensitivity as a cause of bone necrosis and loosening of the prosthesis in total joint replacement. *J. Bone Jt. Surg.* 56-B, *626-642.*

Fearn, B., H. C. Burbidge and G. Bentley (1972). The effect of methylmethacrylate cement on the blood pressure. *Acta Orthop. Scand.* 43, *318-324.*

Feith, R. and T. J. J. H. Slooff (1975). *Experiments in rabbits with a modified acrylic cement. A preliminary report.* Paper read at the 7th International Biomaterials Symposium, April 26-30, 1975, Clemson, U.S.A.

Feith, R., T. J. J. H. Slooff, I. Kazem and Th. J. G. van Rens (1975). Strontium ^{87m}Sr bone scanning for the evaluation of total hip replacement. *J. Bone Jt. Surg. Brit. ed.* in press.

Foster, L. N., R. P. Kelly and W. M. Watts (1951). Experimental infarction of bone and bone marrow. Sequelae of severance of the nutrient artery and stripping of periosteum. *J. Bone Jt. Surg.* 33-A, *396-406.*

Frost, H. M. (1969). Tetracycline-based histological analysis of bone remodelling. *Calc. Tiss. Res.* 3, *211-237.*

Göthman, L. (1960a). The normal arterial pattern of the rabbit's tibia. A microangiographic study. *Acta Chir. Scand.* 120, *201-210.*

Göthman, L. (1960b). The arterial pattern of the rabbit's tibia after the application of an intramedullary nail. A microangiographic study. *Acta Chir. Scand.* 120, *211-219.*

Griss, P., H. von Andrian-Werburg, B. Krempien and G. Heimke (1973). Biological activity and histocompatibility of dense Al_2O_3/MgO ceramic implants in rats. *J. Biomed. Res. Symp.* 4, *453-462.*

Grünert, A. and G. Ritter (1974). Veränderungen physikalischer Eigenschaften der sogenannten Knochenzemente nach Beimischung von Fremdsubstanzen. *Arch. orthop. Unfall-Chir.* 78, *336-342.*

Gustilo, R. B., G. E. Nelson, A. Hamel and J. H. Moe (1964). The effect of intramedullary nailing on the blood supply of the diaphysis of long bones in mature dogs. *J. Bone Jt. Surg.* 46-A, *1362-1363.*

Haas, S. S., G. M. Brauer and G. Dickson (1975). A characterization of polymethylmethacrylate bone cement. *J. Bone Jt. Surg.* 57-A, *380-391.*

Harris, W. H. (1960). A microscopic method of determining rates of bone growth. *Nature* 188, *1038-1039.*

Harris, W. H., R. H. Jackson and J. Jowsey (1962). The in vivo distribution of tetracyclines in canine bone. *J. Bone Jt. Surg.* 44-A, *1308-1320.*

Harris, W. H., D. F. Travis, U. Friberg and E. Radin (1964). The in vivo inhibition of bone formation by alizarin Red S. *J. Bone Jt. Surg.* 46-A, *493-508.*

Heikel, H. V. A. (1960). On ossification and growth of certain bones of the rabbit; with a comparison of the skeletal age in the rabbit and in man. *Acta Orthop. Scand.* 29, *171-184.*

Hench, L. L. and H. A. Paschall (1974). Histochemical responses at a biomaterial's interface. *J. Biomed. Mater. Res. Symp.* 5 (part 1), *49-64.*

Hendriks, J. H. C. L. (1975). Radiologische bijdrage bij het volgen van dialysepatienten. *Ned. Tijdschr. v. Geneesk.* 119, *341-342.*

Herndon, J. H., Ch. O. Bechtol and D. P. Crickenberger (1974). Fat embolism during .total hip replacement. A prospective study. *J. Bone Jt. Surg.* 56-A, *1350-1362.*

Hessert, G. R. and G. Ruckdeschel (1970). Antibiotische Wirksamkeit von Mischungen des Polymethylmethacrylates mit Antibiotica. *Arch. orthop. Unfall-Chir.* 68, *249-254.*

Hessert, G. R. (1971). Bruchfestigkeit und Struktur des Knochenzementes Palacos nach Zusatz von Gentamycin-Sulfat. *Arch. orthop. Unfall-Chir.* 69, *289-299.*

Hirsch, C. (1974). Clinical problems in total hip replacements. *J. Biomed. Mat. Res. Symp.* 5 (part 2), *227-244.*

Holland Ch. J., K. C. Kim, M. I. Malik and M. A. Ritter (1973). A histologic and hemodynamic study of the toxic effects of monomeric methylmethacrylate. *Clin. Orthop.* 90, *262-270.*

Homsy, Ch. A., H. S. Tullos, M. S. Anderson, N. M. Diferrante and J. W. King (1972a). Some physiological aspects of prosthesis stabilization with acrylic polymer. *Clin. Orthop.* 83, *317-328.*

Homsy, Ch. A., Th. E. Cain, F. B. Kessler, M. S. Anderson and J. W. King (1972b). Porous implant systems for prosthesis stabilization. *Clin. Orthop.* 89, *220-235.*

Homsy, Ch. A. (1973). Some mechanical aspects of methylmethacrylate prosthesis seating compound. In: *The hip, pp. 156-163. Proceedings of the first open scientific meeting of The Hip Society, 1973.* C.V. Mosby comp., Saint Louis, U.S.A.

Hulbert, S. F., F. W. Cooke, J. J. Klawitter, R. B. Leonard, B. W. Sauer, D. D. Moyle and H. B. Skinner (1973). Attachment of prostheses to the musculo-skeletal system by tissue ingrowth and mechanical interlocking. *J. Biomed. Mater. Res. Symp.* 4, *1-23.*

Hulbert, S. F., J. R. Matthews, J. J. Klawitter, B. W. Sauer and R. B. Leonard (1974). Effect of stress on tissue ingrowth into porous aluminum oxide. *J. Biomed. Mater. Res. Symp.* 5 (part 1), *85-97.*

Hulliger, L. (1962). Untersuchungen über die Wirkung von Kunstharzen (Palacos und Ostamer) in Gewebekulturen. *Arch. orthop. Unfall-Chir.* 54, *581-588.*

Hupfauer, W. and L. Ulatowski (1971). Thermografische Messungen der Polymerisationstemperaturen thermoplastischer Kunststoffe. *Arch. orthop. Unfall-Chir.* 70, *70-82.*

Hupfauer, W. (1973). Volumenmessungen an Knochenzementen. *Act. Traumat.* 3, *259-262.*

Iida, M., K. Furuya, S. Kawachi, E. Masuhara and J. Tarumi (1974). New improved bone cement (MMA-TBB). *Clin. Orthop.* 100, *279-286.*

Jackson, C. T. and R. M. Greendyke (1965). Pulmonary and cerebral fat embolism after closed-chest cardiac massage. *Surg. Gynec. Obstet.* 120, *25-27.*

Jackson, R. W. and I. MacNab (1959). Fractures of the shaft of the tibia: a clinical and experimental study. *Am. J. Surg.* 97, *543-557.*

Jaffe, W. L., R. M. Rose and E. L. Radin (1974). On the stability of the mechanical properties of self-curing acrylic bone cement. *J. Bone Jt. Surg.* 56-A, *1711-1714.*

Jäger, M., W. Küsswetter, J. Rütt, M. Ungethüm and R. Burckhardt (1974). Experimentelle Torsionslockerung technisch verschieden implantierter Hüftendoprothesenpfannen. *Z. Orthop.* 112, *34-44.*

Jefferis, C. D. (1971). Avoidable complication of McKee-Farrar arthroplasty. *Brit. Med. J.* 2, *382.*

Johnson, R. W. (1927). A physiological study of the blood supply of the diaphysis. *J. Bone Jt. Surg.* 9, *153-184.*

Judet, J., R. Judet, J. Lagrange, J. Dunoyer (1952). *Résection-reconstruction de la hanche. Arthroplastie par prothese acrylique.* Expansion scientifique française, Paris.

Kallos, T., J. E. Enis, F. Gollan and J. H. Davis (1974). Intramedullary pressure and pulmonary embolism of femoral medullary contents in dogs during insertion of bone cement and a prosthesis. *J. Bone Jt. Surg.* 56-A, *1363-1367.*

Kelly, P. J. (1968a). Anatomy, physiology and pathology of the blood supply of bones. *J. Bone Jt. Surg.* 50-A, *766-783.*

Kelly, P. J. (1968b). Effect of unilateral increased venous pressure on bone remodelling in canine tibia. *J. Lab. Clin. Med.* 72/3, *410-418.*

Kim, K. C. and M. A. Ritter (1972). Hypotension associated with methylmethacrylate in total hip arthroplasties. *Clin. Orthop.* 88, *154-160.*

Knappmann, J. (1974). Untersuchungen über die lokale tuberkulostatische Wirksamkeit der Streptomycins nach Beimengung zu Palacos. *Z. Orthop.* 112, *998-1000.*

Koch, H., H. O. Dustmann and K.-P. Schulitz (1972). Die Prophylaxe der Fettembolie beim Hüftgelenkersatz durch Cholinphospholipide und Druckentlastung. Tierexperimentelle Untersuchung. *Arch. orthop. Unfall-Chir.* 72, *272-276.*

Koekenberg, L. J. L. (1963). *Vascularisation in the healing of fractures.* Thesis, Amsterdam. Thomas, Springfield, Ill.

Kölbel, R. and U. Boenick (1972). Mechanische Eigenschaften der Verbindung zwischen spongiösem Knochen mit Polymethylmethacrylat bei statischer Belastung. I. Zugfestigkeit. *Arch. orthop. Unfall-Chir.* 73, *89-97.*

Kölbel, R., U. Boenick, W. Krieger and R. Wilk (1973). Mechanische Eigenschaften der Verbindung zwischen spongiösem Knochen mit Polymethylmethacrylat bei statischer Belastung. II. Scherfestigkeit. *Arch. orthop. Unfall-Chir.* 77, *339-347.*

Koschmieder, R., W. Ritzerfeld and H. Kleymann (1973). Infektionsprophylaxe beim alloplastischen Gelenkersatz durch Gentamycinzusatz zum Polymethylmethacrylat. Tierexperimentelle Untersuchungen. *Z. Orthop.* 111, *244-249.*

Koschmieder, R., W. Ritzerfeld and L. Hohmeyer (1975). Gentamycinzusatz zum Polymethylmethacrylat zur Behandlung von Knocheninfektionen. *Z. Orthop.* 113, *147-149.*

Küntscher, G. (1940). Die Marknagelung von Knochenbrüchen. *Langenbeck's Arch. f. klin. Chir.* 200, *443-455.*

Küntscher, G. (1962). *Praxis der Marknagelung.* F.-K. Schattauer-Verlag, Stuttgart.

Kutzner, F., E. Ch. Dittmann and J. Ohnsorge (1974a). Restmonomerabgabe von abhärtendem Knochenzement. Experimentelle Untersuchungen. *Arch. orthop. Unfall-Chir.* 79, *247-253.*

Kutzner, F., E. Ch. Dittmann and J. Ohnsorge (1974b). Atemeffekte durch Knochenzement auf Methylmethacrylatbasis. Tierexperimentelle Untersuchungen. *Z. Orthop.* 112, *1053-1062.*

Labitzke, R. and M. Paulus (1974). Intraoperative Temperaturmessungen in der Hüftchirurgie während der Polymerisation des Knochenzementes Palacos. *Arch. orthop. Unfall-Chir.* 79, *341-346.*

Langer, K. (1876). Über des Gefässsystem der Röhrenknochen, mit Beiträgen zur Kenntnis des Baues und der Entwicklung des Knochengewebes. *Denkschr. Akad. Wiss., Wien* 36, *1-40.*

Larson, R. L., P. J. Kelly, J. M. Janes and L. F. A. Peterson (1961). Suppression of the periosteal and nutrient blood supply of the femora of dogs. A histologic, microangiographic and roentgenologic study. *Clin. Orthop.* 21, *217-225.*

Lautenschlager, E. P., B. K. Moore and C. M. Schoenfeld (1974). Physical characteristics of setting of acrylic bone cements. *J. Biomed. Mater. Res. Symp.* 5 (part 1), *185-196.*

Lavorgna, J. J., N. A. Burstein, A. L. Schiller and W. H. Harris (1972). The carcinogenesis of plastics used in orthopedic surgery. An assessment of the incidence in rats and the possible relevance to man. *Clin. Orthop.* 88, *223-227.*

Leblond, C. P., G. W. Wilkinson, L. F. Bélanger and J. Robichon (1950). Radioautographic visualization of bone formation in the rat. *Am. J. Anatomy* 86, *289-341.*

Lee, A. J. C., R. S. M. Ling and J. D. Wrighton (1973). Some properties of polymethylmethacrylate with reference to its use in orthopedic surgery. *Clin. Orthop.* 95, *281-287.*

Lee, A. J. C. and R. S. M. Ling (1975). Further studies of monomer lost by evaporation during the preparation of acrylic cement for use in orthopaedic surgery. *Clin. Orthop.* 106, *122-125.*

Lehnartz, E. (1959). *Einführung in die Chemische Physiologie.* Springer, Berlin.

Lexer, E., P. Kuliga and W. Turk (1904). *Untersuchungen über Knochenarterien mittelst Roentgenaufnahmen injizierter Knochen und ihre Bedeutung für einzelne pathologische Vorgänge am Knochensysteme.* A. Hirschwald, Berlin.

Lindwer, J. (1972). *Enige biologische aspecten bij de verankering van de kunstheup. (The behaviour of bone-tissue supporting a hip arthroplasty).* Thesis, Amsterdam.

Lindwer, J. and A. van den Hooff (1975). The influence of acrylic cement on the femur of the dog. A histological study. *Acta Orthop. Scand.* 46, *657-671.*

Lipman, B. S. and E. Massie (1964). Clubbed fingers and hypertrophic osteo-arthropathy. In: *Signs and Symptoms. Applied pathologic physiology and clinical interpretation. (C. M. MacBryde ed.) 4th edition, chapter 11, pp. 246-261.* J. B. Lippincott, Philadelphia.

Lundskog, J. (1972). Heat and bone tissue. An experimental investigation of the thermal properties of bone tissue and threshold levels for thermal injury. *Scand. J. Plast. Reconstr. Surg.,* Suppl. 9.

MacAuley, G. O. (1958). The blood supply of the rat's femur in relation to the repair of cortical defects. *J. Anat.* 92, *665.*

MacNab, I. (1957). Blood supply of the tibia. *J. Bone Jt. Surg.* 39-B, *799.*

MacNab, I. (1958). The blood supply of tubular and cancellous bone. *J. Bone Jt. Surg.* 40-A, *1433-1434.*

Marks, K. E., C. L. Nelson, J. Schwartz, E. P. Lautenschlager and G. W. Marshall (1975). *Characteristics of acrylic bone cements impregnated with antibiotics.* Paper read at the 7th International Biomaterials Symposium, April 26-30, 1975, Clemson, U.S.A.

Marneffe, R. de (1951). Recherches morphologiques et expérimentales sur la vascularisation osseuse. *Acta Chir. Belg.* 50, *469-475, 568-599, 681-701.*

Marsman, A. J. W., P. J. Klopper and J. M. Greep (1975). Acrylbeencement (ABC) en circulatiestoornissen. *Ned. Tijdschr. v. Geneesk.* 119, *856-857.*

Masshoff, W. and A. Neuhaus-Vogel (1974). Die Gelenkkapsel nach Alloplastik. *Arch. orthop. Unfall-Chir.* 78, *175-198.*

Matthews, L. S. and C. Hirsch (1972). Temperatures measured in human cortical bone when drilling. *J. Bone Jt. Surg.* 54-A, *297-308.*

McKee, G. K. and J. Watson-Farrar (1966). Replacement of arthritic hips by the McKee-Farrar prosthesis. *J. Bone Jt. Surg.* 48-B, *245-259.*

McLaughlin, R. E., C. A. DiFazio, M. Hakala, B. Abott, J. A. MacPhail, W. Mack and D. E. Sweet (1973). Blood clearance and acute pulmonary toxicity of methylmethacrylate in dogs after simulated arthroplasty and intravenous injection. *J. Bone Jt. Surg.* 55-A, *1621-1628.*

McLean, F. C. and A. M. Budy (1964). *Radiation, isotopes and bone.* Academic Press, New York.

McMaster, W. C., G. Bradley and Th. R. Waugh (1974). Blood pressure lowering effect of methylmethacrylate monomer. Potentiation of blood volume deficit. *Clin. Orthop.* 98, *254-257.*

Meema, H. E., S. Rabinovich, S. Meema, G. J. Lloyd and D. G. Oreopoulos (1972). Improved radiological diagnosis of azotemic osteodystrophy. *Radiology* 102, *1-10.*

Meyer, P. R., E. P. Lautenschlager and B. K. Moore (1973). On the setting properties of acrylic bone cement. *J. Bone Jt. Surg.* 55-A, *149-156.*

Milch, R. A., D. P. Rall and J. E. Tobic (1958). Fluorescence of tetracyline antibiotics in bone. *J. Bone Jt. Surg.* 40-A, *897-910.*

Milne, I. S. (1973). Hazards of acrylic bone cements. A report of two cases. *Anaesthesia* 28, *538-543.*

Mital, M. and J. Cohen (1966). Repair of experimental bony intramedullary injuries varying in degree. *Surgical Forum* 17, *451-452.*

Modig, J., S. Olerud and P. Malmberg (1973a). Sudden pulmonary dysfunction in prosthetic hip replacement surgery. A case report. *Acta Anaesth. Scand.* 17, *276-282.*

Modig, J., S. Olerud, P. Malmberg and Chr. Busch (1973b). Medullary fat embolization during hip replacement surgery: a preliminary report. *Injury* 5, *161-164.*

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

Müller, M. E. (1962). Die Verwendung von Kunstharzen in der Knochenchirurgie. *Arch. orthop. Unfall-Chir.* 54, *513-522.*

Müller, M. E. (1974a). Der derzeitige Stand der Totalendoprothese des Hüftgelenkes. *Z. Orthop.* 112, *933-938.*

Müller, M. E. (1974b). Late complications of total hip replacement. In: *The hip (W. H. Harris ed.) pp. 319-327. Proceedings of the second open scientific meeting of The Hip Society.* C. V. Mosby comp., Saint Louis, U.S.A.

Nelson, G. G., P. J. Kelly, F. A. Lowell, L. F. A. Peterson and J. M. Janes (1960). Blood supply of the human tibia. *J. Bone Jt. Surg.* 42-A, *625-636.*

Oest, O. (1973). Mechanical strength of the cement-bone-bond. In: *Arthroplasty of the hip (G. Chapchal ed.), pp. 4-8. Proceedings of the 5th International Symposium, January 27-29, 1972, Nijmegen, Netherlands.* G. Thieme Publishers, Stuttgart, Germany.

Oest, O. and K. Müller (1973). Langzeitfestigkeitsuntersuchungen der 3 Knochenzemente CMW, Palacos R und Surgical Simplex. *Act. Traumat.* 3, *269-274.*

Oest, O., W. Hupfauer and K. Müller (1975). *Die Knochenzemente.* F. Enke Verlag, Stuttgart.

Ohnsorge, J. and G. Goebel (1970). Die Verwendung unterkühlter Metallendoprothesen in der Hüftchirurgie. Experimentelle Untersuchungen. *Z. Orthop.* 107, *683-696.*

Ohnsorge, J. and J. Grötz (1974). Kurzzeitige und langzeitige Dimensionsänderung des aushärtenden Knochenzementes. *Z. Orthop.* 112, *975-977.*

O'Leary, R. K. and W. L. Guess (1968). Toxicological studies on certain medical grade plastics sterilized by ethylene oxide. *J. Pharm. Sci.* 57, *12-17.*

O'Leary, R. K., W. D. Watkins and W. L. Guess (1969). Comparative chemical and toxicological evaluation of residual ethylene oxide in sterilized plastics. *J. Pharm. Sci.* 58, *1007-1010.*

Olerud, S., G. Danckwardt-Lilliestrom and G. L. Lorenzi (1969). Do medullary components appear in the femoral vein during the reaming of the tibia? *Europ. Surg. Res.* 1/3, *243-244.*

Olerud, S. and G. L. Lorenzi (1970). Triple fluorochrome labelling in bone formation and bone resorption. *J. Bone Jt. Surg.* 52-A, *274-278.*

Olerud, S. and G. Danckwardt-Lilliestrom (1971). Fracture healing in compression osteosynthesis. An experimental study in dogs with an avascular, diaphyseal, intermediate fragment. *Acta Orthop. Scand.* Suppl. 137.

Ott, G. (1970). *Fremdkörpersarkome.* Exp. Medizin, Pathologie und Klinik, Band 32. Springer-Verlag, Berlin.

Owen, M., J. Jowsey and J. Vaughan (1955). Investigation of the growth and structure of the tibia of the rabbit by microradiographic and autoradiographic techniques. *J. Bone Jt. Surg.* 37-B, *324-342.*

Pahuja, K., H. Lowe and K. Chand (1974). Blood methyl methacrylate levels in patients having prosthetic joint replacement. *Acta Orthop. Scand.* 45, *737-741.*

Patterson, F. P. and C. S. Brown (1972). The McKee-Farrar total hip replacement. Preliminary results and complications of 368 operations performed by five general hospitals. *J. Bone Jt. Surg.* 54-A, *257-274.*

Peebles, D. J., R. H. Ellis, S. D. K. Stride and B. R. J. Simpson (1972). Cardiovascular effects of methylmethacrylate cement. *Brit. Med. J.* 1, *349-351.*

Pegum, J. S. and F. A. Medhurst (1971). Contact dermatitis from penetration of rubber gloves by acrylic monomer. *Brit. Med. J.* 2, *141-143.*

Peters, W. J., R. W. Jackson, K. Iwano and D. C. Smith (1972). The biological response to zinc polyacrylate cement. *Clin. Orthop.* 88, *228-233.*

Philips, H., A. W. F. Lettin and P. V. Cole (1973). Cardiovascular effects of implanted acrylic cement. *J. Bone Jt. Surg.* 55-B, *210.*

Puhl, W. and K.-P. Schulitz (1971). Morphologische Untersuchung über die Polymerisation von Knochenzement. *Arch. orthop. Unfall-Chir.* 69, *300-314.*

Rahn, B. A. and S. M. Perren (1970). Calcein Blue as a fluorescent label in bone. *Experientia* 26, *519.*

Rahn, B. A. and S. M. Perren (1971). Xylenol Orange, a fluorochrome useful in polychrome sequential labeling of calcifying tissues. *Stain Technol.* 46, *125-129.*

Rahn, B. A. and S. M. Perren (1972). Alizarinkomplexon-Fluorochrom zur Markierung von Knochen- und Dentinanbau. *Experientia* 28, *180.*

Rahn, B. A. (1973). *Die polychrome Sequenzmarkierung des Knochens.* Paper read at the Callus-Symposium, Deutsche Gesellschaft der Naturforscher Leopoldina, Leipzig, December, 2-6th, 1973.

Rens, Th. J. G. van (1973). Kritische beschouwingen van de resultaten en complicaties bij honderd totale heupgewrichtsarthroplastieken. *Ned. Tijdschr. v. Geneesk.* 117, *505-506.*

Rhinelander, F. W. and R. A. Baragry (1962). Microangiography in bone healing. I. Undisplaced closed fractures. *J. Bone Jt. Surg.* 44-A, *1273-1298.*

Rhinelander, F. W. (1965). Some aspects of the microcirculation of healing bone. *Clin. Orthop.* 40, *12-16.*

Rhinelander, F. W., R. V. Gracilla, R. S. Philips and W. M. Steel (1967). Microangiography in bone healing. III. Osteotomies with internal fixation. *J. Bone Jt. Surg.* 49-A, *1006-1007.*

Rhinelander, F. W., R. S. Philips, W. M. Steel and J. C. Beer (1968a). Microangiography in bone healing. II. Displaced closed fractures. *J. Bone Jt. Surg.* 50-A, *643-662.*

Rhinelander, F. W. (1968b). The normal microcirculation of diaphyseal cortex and its response to fracture. *J. Bone Jt. Surg.* 50-A, *784-800.*

Rhinelander, F. W. (1972). Circulation in bone. In: *The biochemistry and physiology of bone (G. H. Bourne, ed.), vol. II, chapter 1, pp. 1-77,* Academic Press, New York.

Rhinelander, F. W. (1973). Effects of medullary nailing on the normal blood supply of diaphyseal cortex. In: *The American Academy of Orthopaedic Surgeons. Instructional course lectures, vol. XXII, pp. 161-187.* C.V. Mosby comp., St. Louis, U.S.A.

Rhinelander, F. W. (1974). Tibial blood supply in relation to fracture healing. *Clin. Orthop.* 105, *34-81.*

Richany, S. F., H. Sprinz, K. Kraner, J. Ashby and T. C. Merrill (1965). The role of the diaphyseal medulla in the repair and regeneration of the femoral shaft in the adult cat. *J. Bone Jt. Surg.* 47-A, *1565-1584.*

Rietz, K.-A. (1968). Polymer osteosynthesis III. Segmental resection of femur and fixation of endoprosthesis with methyl methacrylate in dogs. *Acta Chir. Scand.* Suppl. 388.

Ritter, G., A. Grünert and C.-H. Schweikert (1973). Biomechanische Ursachen von Lockerung und Bruch der Hüftendoprothesen. *Arch. orthop. Unfall-Chir.* 77, *154-164.*

Rogers, W. M. and H. Gladstone (1950). Vascular foramina and arterial supply of the distal end of the femur. *J. Bone Jt. Surg.* 32-A, *867-874.*

Roggatz, J. (1974). Das Verhalten der Monomere beim Abbinden von Knochenzement. *Z. Orthop.* 112, *970-972.*

Romeis, B. (1968). *Mikroskopische technik.* R. Oldenbourg Verlag, München.

Ruckdeschel, G., G. R. Hessert and Th. Schöllhammer (1973). Quantitative vitro-Untersuchungen zur Frage der Gentamycinabgabe aus Polymethylmethacrylat-Polymerisatblöcken. *Arch. orthop. Unfall-Chir.* 74, *291-300.*

Salenius, P. and L. E. Laurent (1973). Experience with the McKee-Farrar total hip replacement. A report of 143 operations. *Acta Orthop. Scand.* 44, *451-459.*

Salvati, E. A., R. H. Freiberger and P. D. Wilson (1971). Arthrography for complications of total hip replacement. A review of thirty-one arthrograms. *J. Bone Jt. Surg.* 53-A, *701-709.*

Salvati, E. A. and P. D. Wilson (1973). Long-term results of femoral-head replacement. *J. Bone Jt. Surg.* 55-A, *516-524.*

Schenk, R. and H. Willenegger (1963). Zum histologischem Bild der sogenannten Primärheilung der Knochenkompakta nach experimentellen Osteotomien am Hund. *Experientia* 19, *593-595.*

Schenk, R., Merz, W. A. and Müller, J. (1969). A quantitative histological study on bone resorption in human cancellous bone. *Acta Anat.* 74, *44-53.*

Schlag, G., E. Dingeldein, G. Weisse, H. Regele and St. von Sommoggy (1973). *Tierexperimentelle Untersuchungen mit Knochenzementen.* Paper presented at the First International Congress on prosthetics techniques and functional rehabilitation, March 19-24, 1973, Vienna, Austria.

Semlitsch, M., A. Vogel and H. G. Willert (1971). Kombination moderner Mikroanalysemethoden zur Untersuchung von Gelenkendoprothesen-Abrieb im Bindegewebe der Gelenkkapsel. *Acta Medico technica,* 19, *6: 38-41 and 7: 49-52.*

Semlitsch, M. (1973). Gewebstoxizität der Knochenzemente CMW, Palacos, Simplex und Sulfix an Zellkulturen. *Mitteilung* Nr. 786 Sulzer Brothers Ltd., Winterthur, Switzerland.

Semlitsch, M. (1975). Personal communication.

Shim, S. S. (1968). Physiology of blood circulation of bone. *J. Bone Jt. Surg.* 50-A, *812-824.*

Shim, S. S., D. H. Copp and F. P. Patterson (1970). 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 Jt. Surg.* 50-B, *178-183.*

Sim, F. H. and P. J. Kelly (1970). Relationship of bone remodelling, oxygen consumption and blood flow in bone. *J. Bone Jt. Surg.* 52-A, *1377-1389.*

Slooff, T. J. J. H. (1969). A cement syringe. *Acta Orthop. Belg.* 35, *1012-1014.*

Slooff, T. J. J. H. (1970). *De invloed van het acrylcement bij de fixatie van de heupendoprothese (The influence of the acrylic cement on the fixation of the hipendoprosthesis). A clinical, theoretical, histological and mechanical study.* Thesis, Nijmegen.

Slooff, T. J. J. H. (1971). The influence of acrylic cement. An experimental study. *Acta Orthop. Scand.* 42, *465-481.*

Slooff, T. J. J. H. (1972). Experiments with acrylic cement. In: *Orthopaedic Surgery and Traumatology. Proc. 12th Congress of the S.I.C.O.T., Tel Aviv, October 9-12, 1972.* Excerpta Medica, Amsterdam.

Smith, D. C. and M. E. D. Bains (1956). The detection and estimation of residual monomer in polymethylmethacrylate. *J. Dent. Res.* 35, *16-24.*

Smith, D. C. (1968). A new dental cement. *Br. Dent. J.* 125, *381-384.*

Smith, D. C. (1974). Materials used for construction and fixation of implants. *Oral Sci. Rev.* 5, *23-55.*

Suzuki, H. E. and A. Mathews (1966). Two-colour fluorescent labeling of mineralizing tissues with tetracycline and 2,4-bis [n,n'-di(carbomethyl aminomethyl] fluorescein. *Stain Technol.* 41, *57-60.*

Szyszkowitz, R. (1971). Einbau und Abbau von Knochenzement bei Kombinations-Osteosynthesen im Tierversuch. *Arch. orthop. Unfall-Chir.* 71, *71-94.*

Thompson, J. R. and S. D. Entin (1969). Primary extraskeletal chondrosarcoma. Report of a case arising in conjunction with extrapleural lucite ball plombage. *Cancer,* 23, *936-939.*

Tilling, G. (1958). The vascular anatomy of long bones. A radiological and histological study. *Acta Radiologica,* Suppl. 161.

Tronzo, R. G., T. Kallos and M. Q. Wyche (1974). Elevation of intramedullary pressure when methylmethacrylate is inserted in total hip arthroplasty. *J. Bone Jt. Surg.* 56-A, *714-718.*

Trueta, J. and A. X. Cavadias (1955). Vascular changes caused by the Küntscher type of nailing. *J. Bone Jt. Surg.* 37-B, *492-505.*

Trueta, J. (1959). The three types of acute haematogenous osteomyelitis. A clinical and vascular study. *J. Bone Jt. Surg.* 41-B, *671-680.*

Trueta, J. and J. D. Morgan (1960). The vascular contribution to osteogenesis. I. Studies by the injection method. *J. Bone Jt. Surg.* 42-B, *97-109.*

Trueta, J. (1963). The role of the vessels in osteogenesis. *J. Bone Jt. Surg.* 45-B, *402-418.*

Trueta, J. and A. X. Cavadias (1964). A study of the blood supply of the long bones. *Surg. Gynec. Obstet.* 118, *485-498.*

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

Vanderhoeft, P. J., P. J. Kelly, J. M. Janes and L. F. A. Peterson (1963). Growth and structure of bone distal to an arteriovenous fistula: Quantitative analysis of tetracycline-induced transverse growth patterns. *J. Bone Jt. Surg.* 45-B, *582-596.*

Verhofstad, A. A. J., F. E. Schrijer and H. W. J. Joosten (1975). Fluorescence microscopy of large object fields. Description of a microscope with incident illumination adapted for the observation of large object fields. *J. Microsc.* - to be published.

Vilmann, H. (1969). The in vivo staining of bone with alizarin Red S. *J. Anat.* 105, *533-545.*

Wagner, J. and R. Bourgois (1974). *Ageing of methylmethacrylate. Alteration of mechanical characteristics.* Paper read at annual meeting of the Belgian Soc. Orthop. Surg. and Traumatol. on implants in orthopaedic surgery, May 23-26, 1974, Brussels.

Wahlig, H., W. Hameister and A. Grieben (1972). Ueber die Freisetzung von Gentamycin aus Polymethylmethacrylat. *Langenbecks Arch. Chir.* 331, *169-212.*

Wahlig, H. and H. W. Buchholz (1973). Knochenzement und Gentamycin. *Akt. Traumatologie* 3, *247-252.*

Walker, P. S. (1973). The optimum use of materials in total hip design. In: *The hip, pp. 46-66. Proceedings of the first open scientific meeting of The Hip Society, 1973.* C.V. Mosby comp., Saint Louis, U.S.A.

Willert, H.-G. and P. Puls (1972). Die Reaction des Knochens auf Knochenzement bei der Allo-Arthroplastik der Hüfte. *Arch. orthop. Unfall-Chir.* 72, *33-71.*

Willert, H.-G. (1973a). Tissue reactions around joint implants and bone cement. In: *Arthroplasty of the hip (G. Chapchal ed.), pp. 11-21. Proceedings of the 5th International Symposium, January 27-29, 1972, Nijmegen, Netherlands.* G. Thieme, Stuttgart, Germany.

Willert, H.-G. and M. Semlitsch (1973b). Die Reaktion der periartikulären Weichteile auf Verschleissprodukte von Endoprothesenwerkstoffen. In: *Der totale Hüftgelenkersatz (H. Cotta and K.-P. Schulitz ed.), pp. 199-209.* Thieme-Verlag, Stuttgart, Germany.

Willert, H.-G., H.-A. Frech and A. Bechtel (1973c). *Measurements of the quantity of monomer leaching out of acrylic bone cement into the surrounding tissues during the process of polymerization.* 166-th Meeting American Chemical Society in Chicago, U.S.A. (August 26-31, 1973). Symposium on biomedical applications of polymers, paper 52.

Willert, H.-G. and M. Semlitsch (1974a). *Articular capsule reactions associated with joint endoprostheses.* Paper read at the Congress of Dutch-Swiss Orthopaedic Societies, May 1974, Lausanne, Switzerland.

Willert, H.-G., J. Ludwig and M. Semlitsch (1974b). Reaction of bone to methacrylate after hip arthroplasty. A long-term gross, light microscopic, and scanning electron microscopic study. *J. Bone Jt. Surg.* 56-A, *1368-1382.*

Wilson, J. N. and J. T. Scales (1970). Loosening of total hip replacements with cement fixation. Clinical findings and laboratory studies. *Clin. Orthop.* 72, *145-160.*

Wilson, P. D., H. C. Amstutz, A. Czerniecki, E. A. Salvati and D. G. Mendes (1972). Total hip replacement with fixation by acrylic cement. A preliminary report of 100 consecutive McKee-Farrar prosthetic replacements. *J. Bone Jt. Surg.* 54-A, *207-236.*

Wiltse, L. L., R. H. Hall and J. C. Stenchjem (1957). Experimental studies regarding the possible use of self-curing acrylic in orthopaedic surgery. *J. Bone Jt. Surg.* 39-A, *961-972.*

de Wijn, J. R., T. J. J. H. Slooff and F. C. M. Driessens (1974a). Mechanical properties of bone cements in vitro and in vivo. In: *The knee joint (ed. O. S. Ingwersen et al.), pp. 1-5. Proceedings of the International Congress, Rotterdam, september 13-15, 1973.* Excerpta Medica, Amsterdam.

de Wijn, J. R. (1974b). Reduction of maximum temperature in the polymerization of cold- and heatcuring acrylic resins. *J. Biomed. Mater. Res.* 8, *421-434.*

de Wijn, J. R. (1975a). *Polymethylmethacrylate-aqueous phase blends; in situ curing porous materials.* Paper read at the 7th International Biomaterials Symposium, April 26-30, 1975, Clemson, U.S.A.

de Wijn, J. R., T. J. J. H. Slooff and F. C. M. Driessens (1975b). Characterization of bone cements. *Acta Orthop. Scand.* 46, *38-51.*

de Wijn, J. R., F. C. M. Driessens and T. J. J. H. Slooff (1975c). Dimensional behaviour of curing bone cement masses. *J. Biomed. Mater. Res. in press.*

Zagar, L. A. (1972). Determination of residual ethylene oxide in methyl methacrylate polymer powders by GLC. *J. Pharm. Sci.* 61, *1801-1802.*

Zichner, L. (1970). Zur Bedeutung der Spongiosa- und Knochenmarks-Embolie in die Lunge. *Langenbecks Arch. Chir.* 326, *367-379.*

Zucman, J., P. Maurer, C. Berbesson and J. Barbot (1968). Etude expérimentale de l'action ostéogénique des greffes de périoste, des greffes de moelle osseuse et de l'alésage centro-médullaire. *Revue de Chir. Orthop.* 54, *221-238.*