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Hematogenous Infection of Total Joint Replacement

An experimental study in the rabbit

BY

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III: LATE HEMATOGENOUS INFECTION AFTER TOTAL JOINT REPLACEMENT.
Studies of gentamicin and bone cement in the rabbit.
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IV: LATE ANAEROBIC HEMATOGENOUS INFECTION OF EXPERIMENTAL TOTAL JOINT REPLACEMENT.
A study in the rabbit using Propionibacterium acnes.
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ABBREVIATIONS

THR = total hip replacement
TJR = total joint replacement

INTRODUCTION

Long preceded by microbes, the evolution of man has come about in a world where the bacteria have pursued a permanent territorial imperative. As pointed out by Krizek and Robson (1975), infection is a result of a disturbance in the delicate balance man has established with his bacterial environment. This balance is defined as a quantitative relationship and can be altered by either lowering the host defence mechanism or increasing the bacterial number. Intact cutaneous and mucous membrane epithelial barriers are requisite qualifications for defence against the invasion by bacteria.

Wound contamination

All operative wounds are subjected to a certain degree of bacterial contamination. The possible routes for dissemination to the wound tissue are partly exogenous: direct contamination by contact with unsterile objects or by sedimentation of bacteria bearing dust particles in the air; partly endogenous: by contamination of bacteria resident at the edges of the skin, by liberation through entry of bacteria-containing hollow spaces or by contamination from bacteria occurring in the circulation.

In modern hospitals instruments, implants and other necessary equipments could be expected to be delivered totally free of microorganisms to the operating room.

An empty operating theatre with modern air-filters and a ventilatory capacity of 12 to 20 air changes/hour is almost completely free of bacteria in the air (Fitzgerald and Washington 1975). With people in the room, however, contamination takes place. The degree of contamination measured as colony-forming units/m^3 air, is directly

proportional to the number of people present and the degree of activity (Ford et al. 1967, Fitzgerald and Washington 1975, Pollock 1979) and inversely proportional to the number of air changes/time unit (Charnley 1972, Schonholtz 1976).

Preoperative washing and disinfection result in a cutaneous surface free of bacteria (Smith 1979). In the deeper cutaneous layers and in the sebaceous glands bacteria remain and cannot be removed without damage to the tissue. The operative incision allows the escape of these bacteria, and Smith (1979) has theoretically calculated the amount to between 100 and 30,000 organisms in an incision of 15 cm length. Qualitatively the cutaneous resident flora consists mainly of anaerobic bacteria (mostly *Propionibacterium acnes*) and *Staphylococcus epidermidis* (Smith 1979).

Hematogenous contamination of surgical wounds has been studied experimentally in animals and a considerable degree of wound contamination has been reported during bacteremia (Krizek and Davis 1966, Howe 1968 and 1969).

The recovery of bacteria from the wound is dependent on the size of the bacterial population and the culture technique (Nyström 1980). Even with an exquisite technique, a wound contamination consisting of less than 10^3 bacteria/g tissue would probably not be detected.

Transient, usually asymptomatic, episodes of bacteremia have been reported to occur in assocation with various manipulations, instrumentations, and surgical procedures in the patients. Trauma to mucous membranes, particularly, is associated with a high frequency of bacteremia (Everett and Hirschmann 1977). In connection with the surgical procedures there is a risk of bacteremia by manipulations of the genito-urinary tract (Sullivan et al. 1973, Irvine et al. 1974, Donovan et al. 1976, Wroblewski and delSel 1980), the upper air

ways and the mouth (Berry et al. 1973, Rubin et al. 1976) and by the use of intravenous catheters and infusions (Maki et al. 1973, Stjärnström et al. 1978).

Wound infection

The extent of the contamination of the wound is the main determinant for the risk of infection. Elek (1956 and 1957) was able to show that an intradermal or subcutaneous injection required 2-8.5 x 10^6 staphylococci to produce a local abscess in healthy volunteers. Both animal experiments and clinical experience have proved that bacterial numbers exceeding 10^5/g tissue or ml of body fluid result in a high probability of infection. Bacterial numbers below 10^5/g usually result in primarily healing of the wound (Robson, Krizek and Heggers 1973).

The increased risk of infection caused by foreign bodies in the operative wound is clinically well known. Elek and Conen (1957) as well as Edlich et al. (1968) demonstrated experimentally that the figure for the smallest population of bacteria which could cause an infection was lowered by placing a suture in the wound. It could not be shown whether this was achieved by the suture itself or by secondary ischemia and tissue necrosis. Howe (1968), on the other hand, observed in bacteremic animals no infection promoting effect of a silk tie around a small piece of muscle tissue in the operative wounds.

The importance of the local defence mechanisms in the prevention of wound infection has been studied by Miles, Miles and Burke (1957) and Polk and Miles (1973). The primary lodgement of bacteria in the operative wound was subjected to an extensive killing by the local defence mechanisms during the first three to four hours. The outcome was determined by the amount of bacteria that survived this initial decisive period. Local or systemic modifiers resulting in tissue hypoxia during this decisive period resulted in enhancement of infection but was without effect later on. Burke (1961) also demonstrated that the

ability of systemic antibiotics to prevent development of a primary bacterial lesion was confined to this decisive period. Maximal suppression was obtained if the antibiotic was in the tissue when the bacteria arrived.

Total joint replacement and infection

The total joint replacement (TJR) of hip and knee joints is today a procedure so common as to have become routine at most orthopaedic centers. The high risk of infection of TJR is a recognized problem and several investigations during the last 10 years have concerned detailed clinical studies of infection problems in total hip replacements (THR). Replacements of large joints other than the hip have not yet been made in such a number that reliable information in this context is to be found in the literature (Harris 1978). However, since the operative technique of TJR is similar in the large joints, the reported conditions concerning THR, most probably, are universal for TJR. The discussion below is therefore confined to THR.

Since John Rhea Barton in 1826 performed a mobile osteotomy in an ankylotic hip, countless methods have been developed in attempting to treat hip joints injured by disease or trauma. The modern principles of hip replacement evolved during the 1950s and 60s by, among others, McKee, Watson-Farrar, Müller and Charnley. Charnley's contributions, which include the development of the low-friction arthroplasty and the introduction of polymethyl-methacrylate for fixation and load transmission, have been of fundamental importance in the success of THR. The initial enthusiasm about the operative results was cooled down, however, because of the high postoperative infection rate often with cathastrophic consequences for the patient which necessitated extraction of the prosthesis. Charnley (1972) considered that the risk of infection in THR was so serious that even one single infected dust particle would be able to

cause an infectious process.

Several technical factors in the operative procedures of THR are known to favour infection, for instance the long duration of the operation and the exposure of considerable wound surfaces with attendant risk of soft tissue injury and necrosis. Fitzgerald et al. (1977), in a material comprising 3215 THR, found that the incidence of postoperative infection was lower if the operative period was under than if it exceeded 140 minutes (0.9 and 1.7 per cent respectively). The difference, however, was not statistically significant. Hematoma, dead space, decreased wound perfusion, foreign bodies, and necrotic tissue were by Robson, Krizek and Heggers (1973) considered to be the factors in the wound which interfered most with the defence mechanisms on the local level. Hematoma, dead space, and lowered tissue perfusion are factors which vary with every THR and their extent is difficult to estimate. Hematoma is considered to be a definite risk and many reports relate infections directly to the presence of postoperative hematomas (Fitzgerald et al. 1977, Nelson et al. 1980). Charnley, in 1968, developed a special suture technique to minimize the dead space and the hematoma, this has probably contributed to the gradual decrease of infection rates in his material (Charnley 1972). The presence of foreign bodies and necrotic tissue are unavoidable in THR. Willert et al. (1974) stated three causes of the postoperative bone necrosis: (1) damage to the descending metaphyseal arteries during resection of the femoral head and destruction of nutrient arteries during preparation of the methaphyseal and diaphyseal medullary cavity for implantation, (2) heat up to 72°C generated during the polymerization of the methacrylate. and (3) cytotoxic effects of nonpolymerized monomer. He believed that the vascular damage may be expected to cause infarct necrosis in the diaphysis and trochanter, whereas the polymerization heat and the toxic effect of the monomer are most likely responsible for the zonal necrosis. Jefferiss et al.

(1975) and Reckling and Dillon (1977) showed that the osteonecrosis was not obviously dependent on thermal injury. Linder (1976) demonstrated the toxic characteristics of methyl-methacrylate monomer in studies of the microvascular system in the rabbit's ear. Later (1977), by investigations on the rabbit tibia, he showed that the monomer and thermal trauma were not additive to the injury caused by the surgical preparation of the implant bed. Rhinelander et al. (1979) reported a study on the dog which showed that the preparation of the prosthetic bed led to apparent necrosis of the inner zone of cortical bone, especially in the diaphysis. Full recovery was observed in 6 months if the marrow cavity was not filled. When acrylic cement was introduced into the marrow cavity, extensive necrosis was still present after a year, when their study was terminated.

Infection rate and prophylactic procedures of total hip replacement

At an early stage it became evident that THR performed using standard surgical routines and without prophylactic measures resulted in infection rates around five per cent (Charnley 1972, Müller 1974, Murray 1974). Reported frequencies varied between 0 (Chapcal et al. 1973) and 15 per cent (Carlsson et al. 1977). Probably, the differences can be partly explained by definition problems and variations in culture techniques. Bacteria previously regarded as non-pathogenic, such as *Staphylococcus epidermidis*, have also been implicated in the etiology of a large number of infected THR (Nelson 1977). Kamme et al. reported 1974 the presence of anaerobic bacteria in late infections of THR.

Three principles have been developed aimed to lower the postoperative infection rate:

1. Prophylactic systemic antibiotic;
2. Clean air enclosure;
3. Antibiotic impregnated bone cement.

Prophylactic systemic antibiotic

In animal experiments Burke (1961) has shown that the optimal possibility of preventing wound infections was dependent on the presence of the antibiotic in the wound tissue at the time of the bacterial contamination. Thus, prophylaxis against infection would be expected if antibiotics are administered preoperatively. There seems to be a certain risk of a rising incidence of wound infection and of the development of resistant organisms if the antibiotic treatment is started postoperatively (Tachdjian and Compere 1957, Barnes et al. 1959, Olix et al. 1960, Schonholtz et al. 1962, Stevens 1964). Pavel et al.,in 1974, reported a double-blind prospective study involving 1591 clean orthopaedic surgical procedures performed to test the effectiveness of preoperative and intraoperative antibiotics in reducing the postoperative infection rate. The antibiotic chosen for the study was cephaloridine. A statistically significant decrease in the over-all postoperative infection rate from 5 per cent in the placebo group to 2.8 per cent in the antibiotic group was found. Eriksson et al. (1973), also in a double blind study, found a significant reduction in the postoperative infection rate of THR by the prophylactic use of cloxacillin.

Infection rates between 0 and 2 per cent are reported when prophylactic systemic antibiotics are used (table 1).

Clean air enclosure

Both Alexakis et al. (1976) and Franco et al. (1977) found a significantly reduced number of colony-forming units (95 per cent and a factor of 12 respectively) when laminar air flow (LAF) was used. By the introduction of aspirator gowns the number of CFU could be further reduced (by 4 per cent and a factor of 2 respectively). Franco et al. could not demonstrate any reduction in the average number of bacteria in the wound by using LAF, but on the other hand, they found a difference in the median

TABLE 1. Prophylactic systemic antibiotic.

Reported by	Prophylactic antibiotic	Number of THR		Number of infected THR		Frequency (%) of infected THR	
		totally	no previous surgery	totally	no previous surgery	totally	no previous surgery
Murray -73	Cephalosporine or Erythromycin	808	557	12	4	1.5	0.7
Bentley and Duthie -73	Cloxacillin	229	200	3	2	1.3	1.0
Eftekhar et al. -76	Penicillin G or Oxacillin Ampicillin	800		4		0.5	
Collis and Steinhaus -76	Methicillin or Cephalothin	298		0		0	
Salvati -76	Oxacillin	526		8		1.5	
Donovan et al. -76	Cephalosporine	486		6		1.2	
Fitzgerald et al. -77	Methicillin	3215	2224	47	19	1.5	0.9
Carlsson et al. -77	Cloxacillin	695		14		2.0	
Olsson et al. -79	Penicillin G	238	208	0	0	0	0
Pollard et al. -79	Cephaloridine or Flucloxacillin	310	279	4	3	1.3	1.1
		7605	3468	98	28	1.3	0.8

value indicating more wounds without bacterial contamination when LAF was used. There was no correlation between air and wound contamination in their material. Both Alexakis et al. and Franco et al. questioned the importance of the air-borne bacterial contamination in postoperative wound infections. This is supported in a study of THR by Schwan et al. (1977) and Turner et al. (1974) who, in a large material of different orthopaedic procedures found, that the infection rate was 0.8 per cent in 3407 operations with LAF and 1 per cent in 8253 operations without LAF. The earliest and perhaps leading advocate of LAF is Charnley. In his extensive material of THR there is a gradual reduction of the infection rates from well over 7 per cent to less than 1 per cent. This reduction accompanied the introduction of increasingly refined clean air enclosure systems as well as improvements of the surgical technique. The effectiveness

of clean air enclosures in reducing postoperative infection rates is also supported by Salvati (1976) who, using antibiotic prophylaxis, had 8 infected cases in 526 THR (1.5 per cent) and by the introduction of LAF reduced the rate of infection to 12/1249 (1.0 per cent). With clean air enclosure the reported infection rates vary between 0.6 and 1.8 per cent (table 2).

TABLE 2. Clean air enclosure.

Reported by	Number of THR		Number of infected THR		Frequency (%) of infected THR	
	totally	no previous surgery	totally	no previous surgery	totally	no previous surgery
Brady et al. -75	300		3		1.0	
Stühmer et al.- 77	977	629	18	4	1.8	0.6
Charnley -79	5405	4644	33	26	0.6	0.6
van Niekerk and Charnley -79	2154	1718	18	7	0.8	0.4
Nelson et al. -80	243		4		1.6	
	9079	6991	76	37	0.8	0.5

Antibiotic impregnated bone cement

The possibility of using antibiotics mixed in the bone cement as prophylaxis in THR was tested by Buchholz at the end of the 1960s. Gentamicin proved to be superior to other antibiotics and has been used by Buchholz and co-workers in a large number of THR since the beginning of the 1970s (Buchholz and Gartmann 1972). Buchholz (1979) reported infection rates of 0.8, 0.8 and 0.5 per cent for the years 1975, -76 and -77 respectively. The efficiency of gentamicin bone cement has recently been studied in a prospective multicentered study in Sweden in which prophylaxis with systemic antibiotics and gentamicin cement was altered between every second patient.

The infection rate was in the gentamicin group 0.4 per cent (3/831) and in the antibiotic group 1.6 per cent (13/812) (Josefsson et al. 1980).

In revision arthroplastics of infected THR satisfying results have been obtained by the use of gentamicin cement, mostly in combination with systemic antibiotics. Buchholz (1979) reported success in 86 per cent and Carlsson et al. (1978) considered 60/77 (=78 per cent) reoperations to have healed.

Previous surgery

Previous surgery, as osteotomy or nailing of a fracture, have been reported to increase the incidence of infection following THR (Fitzgerald et al. 1977, Stühmer et al. 1977, Nelson et al. 1980, Andrews et al. 1981). This increase is evident both with antibiotic prophylaxis and clean air enclosures (table 1 and 2). The cause of this increase is obscure, but decreased resistance of scar tissue and latent infection have been discussed.

Bacteriology

Around 50 per cent of all infected THR are presented during the first postoperative year (Eftekhar 1974, Hunter and Dandy 1977, Salvati 1976, Amstutz and Kass 1977). Late infections have been reported to occur as late as in the 12th postoperative year (Buchholz 1979). Most authors set the borderline between early and late infections at three months postoperatively. Hunter and Dandy (1977) found no obvious difference in the type of organism cultured from the early infections and that cultured from the late infections. In a review of eight publications, including a total of 392 infected THR, Nelson (1977) found that the etiology was in 60 per cent gram-positive and in 20 per cent gram-negative bacteria. *Staphylococcus aureus* and *Staphylococcus epidermidis* dominated the gram-positive group while *Escherichia coli* and *Proteus* were usually found in the gram-negative infections. Anaerobic bacteria were responsible for 5 per

cent and sterile or non-valid cultures made up 15 per cent of the cases.

In recent years there has been an increasing awareness of the importance of anaerobic bacteria as an etiologic agent especially in late infections of THR. More wide--spread use of anaerobic culture techniques have revealed a considerable number of infections caused by these micro-organisms. Kamme et al. (1974) reported growth of anaerobic bacteria in 4/7 reoperations of THR. Nolan et al. (1975) found that growth of anaerobic bacteria was obtained in 25 per cent of 37 patients reoperated on because of infection. Buchholz et al. (1977) stated anaerobic growth in 95/439 and Carlsson et al. (1978) in 23/77 reoperations. The anaerobic bacteria have mostly been composed of *Peptococcus, Peptostreptococcus,* and *Propionibacterium acnes* (Kamme et al. 1974, Evanski et al. 1977, Kamme and Mårdh 1977. Carlsson et al. 1978, Petrini et al. 1979).

Hematogenous infection of total hip replacements
In 1973 Lazansky and Mallory each reported a case of a hematogenously infected THR associated with pneumonia. In 1974 Irvine et al. drew attention to the relationship between manipulations of the genito-urinary tract and infection of THR. Since then several case reports and papers have given accounts of late infections of THR secondary to distant foci of infection. Table 3 summarizes the bacterial findings and table 4 the primary foci of infection in the cases that the present author has found reported in the literature.

Fitzgerald et al. (1977) reported 42 infections out of a total of 3210 THR. They classified the infected cases in three stages. Stage-I infections (acute fulminating infections) presented within the first three postoperative months and included 17 hips. These were based on peroperative contamination. Stage-II infections (delayed sepsis) included 18 hips and were diagnosed at four to 25 months

TABLE 3. Etiologic bacteria in 61 hematogenously infected total hip replacements (57 patients) reported in the literature 1973 - 1980.

Bacteria	Number	Frequency (%)
Staphylococcus aureus	28	46
β-Streptococcus	9	15
Pneumococcus	5	8
Staphylococcus epidermidis	5	8
Escherichia coli	4	7
Proteus mirabilis	4	7
Pseudomonas	1	10
Mycobacterium tuberculosis	1	
Salmonella typhimurim	1	
Streptococcus viridans and Proteus mirabilis	1	
Bacteroides fragilis	1	
Aeromonas hydrophilia	1	

Reported by: Mallory 1973, Irvine et al. 1974, Artz et al. 1975, Burton and Schurman 1975, Cruess et al. 1975, D'Ambrosia et al. 1976, Donovan et al. 1976, Eftekhar et al. 1976, Rubin et al. 1976, Carlsson et al. 1977, Downes 1977, Fitzgerald et al. 1977. McCollough 1977, Ahlberg et al. 1978, Karlström and Wigren 1978, Lattimer et al. 1979, Josefsson et al. 1980, Stinchfield et al, 1980, Wroblewski and delSel 1980, Øvrum and Dahl 1980.

postoperatively. Ten of these were interpreted as being the result of peroperative contamination, while the backgrounds of eight cases were obscure. Stage-III infections (late hematogenous infections) included seven hips and were diagnosed at 23 to 50 months postoperatively. These were regarded as unmistakeable hematogenous infections. Thus, at least 7/25 (=28 per cent) late infected THR in their material were considered to be hematogenous.

TABLE 4. Primary foci of infection in 61 hematogenously infected total hip replacements in the literature 1973 - 1980.

Primary foci	Number
Respiratory tract	11
Dermal	10
Urinary tract	9
Dental	7
Gastro-enteric tract	4
Otitis media	1
Parotitis	1
Unknown	14

(For references see table 3)

The incidence of hematogenous infection was estimated by Charnley (1972) at 0.3 per cent. This figure is in accordance with that of Ahlberg et al. (1978), who reported hematogenous infection in 5/1716 THR. Many of the patients with hematogenously infected THR have showed an acute and dramatic course with an unusually high mortality rate (Wilson et al. 1975, D´Ambrosia et al. 1976, Salvati 1976). This is probably because pathogenic bacteria, like *Staphylococcus aureus*, were commonly the cause of those cases classified as hematogenous infections.

The most common bacteria in septicemia, as stated by Svanbom (1979), were gram-negative enteric rods (mainly *Escherichia coli*), *streptococci* and *staphylococci* (mainly *Staphylococcus aureus*) followed by *pneumococci*. In Svanbom´s study the portals of entry were chiefly the cutis, the genito-urinary tract, and the respiratory tract (including the ear), while the dental origin was responsible in only 5/151 cases. Wilson et al. (1972), Williams et al. (1976), and Noone et al. (1978) found

that, between 6 and 20 per cent of all the positive blood cultures in their hospitals were due to anaerobic bacteria. Felner (1974) reported 18 cases of endocarditis caused by anaerobic bacteria of which *Propionibacterium acnes* was the most common. Moreover, *Propionibacterium acnes* has been a frequent cause of endocarditis following open-heart surgery (Levin 1966, Johnsson et al. 1968).

The background of the present investigation

Most likely, the reported numbers of hematogenously infected THR represent only partly the total number of those cases. As bacteremia often runs a subclinical course and the primary foci of infection is not always obvious, many cases of hematogenous infections are probably wrongly interpreted as being due to peroperative contamination.

The risk of bacteremia is present both outside and inside the hospital but is greatly increased in association with several different interventions to which the patient is subjected (Everett and Hirschmann 1977). Except in connection with manipulations of the genito-urinary tract (Irvine et al. 1974, Wroblevski and delSel 1980), attention has not been drawn to the occurrence of a hematogenous origin of the early infections of THR.

Many cases of anaerobically infected THR have been diagnosed. These infections are mostly considered to have been caused by peroperative contamination. Since anaerobic bacteremia is fairly frequent (Wilson et al. 1972, Williams et al. 1976, Noone et al. 1978) it seems quite possible that a hematogenous contamination of joint replacements could occur even with these organisms.

There is now considerable clinical experience with the prophylactic use of bone cement with gentamicin and few side effects have been reported (Buchholz and Gartman 1972, Carlsson et al. 1978, Josefsson et al, 1980). The

concentration of gentamicin is high around the implanted cement during the first two-three weeks but there is also a low continuous release and persisting tissue concentration (Wahlig and Buchholz 1972, Wahlig and Dingeldein 1980). Accordingly, bone cement prepared with gentamicin might also be effective in the prevention of a late hematogenous infection.

The purpose of the present experiments was to study:

1. Bacteremia as the cause of postoperative infection (I);
2. Hematogenous dissemination to the cemented total joint arthroplasty (II, III and IV);
3. Prophylactic effect of gentamicin bone cement against hematogenous infection (III).

MATERIALS AND METHODS

The experimental model

The basic idea was to create a model for human total joint replacements using animals suitable for experimental work in order to study the dissemination of intravenously injected bacteria to these artificial joints. To resemble the human clinical conditions, the experimental total joint replacement would have to fulfill the following conditions:

1. Prostheses consisting of two articulating parts made from high density polyethylene and metal (Vitallium or stainless steel).
2. Fixation of the prostheses by self-curing acrylic cement (polymethylmetacrylate).
3. A major joint as replacement site. Resection of the normal articulating surfaces and fixation of both parts within the metaphyseal and diaphyseal areas of the bone.

The prosthesis. Some of the endoprostheses designed for replacement of human finger joints meet the demands listed above. St. Georg Fingermittelgelenk Endoprothese (Waldemar Link) were used in all cases but one. In that case a Steffee MP joint prosthesis (DePuy) was inserted (in Experiment II). The prostheses consist of a polyethylene and a Cobalt-Chrome-Molybdenum alloy component. Both components have long stems which are designed for introduction into the diaphyseal part of the bone (fig.1).

The animal. Rabbits were used as experimental animals because they have proved to be suitable for experiments concerning osteomyelitis and septic arthritis (Thompson and Dubos 1938, Rigdon 1942, Norden 1970, Schurman 1978, Marks 1980). The knee joints of rabbits are of a size

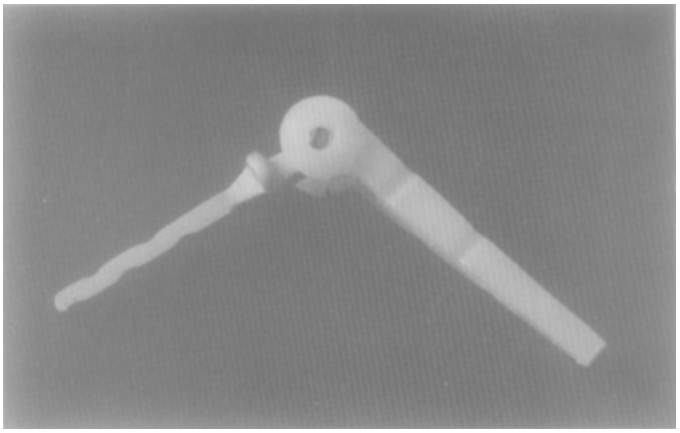

Fig. 1. The St. Georg Fingermittelgelenk Endoprothese used in the present Experiments II, III and IV (see list on page 3).

almost equal to human finger joints. The St. Georg prosthesis fits excellently in the rabbit knee joint.

For these experiments a total number of 68 male New Zealand white rabbits were used (table 5). The animals were fed a commercial maintenance diet (Ewos AB. Södertälje. Sweden) and were given water *ad lib*. They were individually housed in cages in a room with 12 hours of day light, a temperature of 18-20°C, and relative humidity of 45-55 per cent.

Bacteria

Staphylococcus aureus (Wood 46) was used in the studies reported in Papers I, II and III. The strain was chosen because of its

1) capability of causing bone and joint infections (Rigdon 1942, Lindberg 1969, Norden 1970, Andriole et al. 1973, Elson et al. 1977);

2) importance as an etiologic factor in infection of THR (Nelson 1977);

3) characteristic pattern identifiable by desoxyribonuclease and coagulase production, and phage typing.

Propionibacterium acnes (ATCC 6919) was used in the study reported in Paper IV. This agent was chosen because of its

1. known potentiality to cause osteomyelitis (Lewis et al. 1978, Raff and Melo 1978)
2. ability to cause late infection of THR (Carlsson et al. 1978, Buchholtz 1979);
3. characteristic pattern identifiable by biochemical methods and phage typing.

Anesthesia

Forty-five of the rabbits were given an intramuscular combination of 100-150 mg of pentobarbital (Nembutal^{R}, Abbot) and 50-100 mg of ketamine chloride (Ketalar^{R}, Parke Davis). The rest had a neurolept analgesia with fentanyl-fluanison (Hypnorm^{R}, Leo. Sweden) administrated intramuscularly in a dose of 0.5-0.8 ml per kg body-weight.

Surgery

A modern operating room, disposable sterile dresssings including sterile adhesive drapes, and a sterilized set of instruments for each rabbit were used. The operative area was depilated with an electrical razor and desinfected by soaking the extremity with chlorhexidin-alcohol. All wounds were closed with polyglycolic acid sutures (Dexon^{R}, Davis + Geck).

Experiment I: A longitudinal incision exposed the lateral aspect of the proximal femur. A fenestration of approximately 3 x 10 mm was performed distal to the greater trochanter bilaterally. On the right side the defect was filled with acrylic bone cement (CMW Laboratories Ltd), on the left side no further procedures were performed.

Experiment II: Both knee joints were approached through a medial parapatellar incision. The patella was dislocated laterally. On the right side the joint surfaces were resected and the medullary canals were opened. Commercial

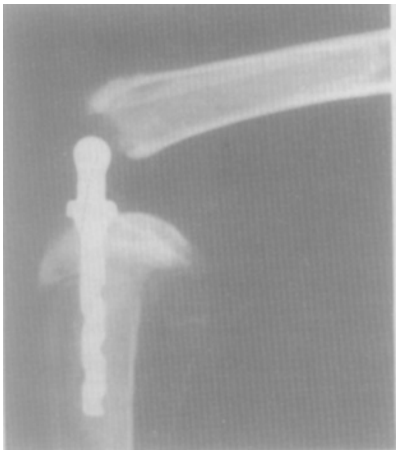

Fig. 2. Radiograph of a specimen in Experiment II (see list on page 3) with the experimental total joint replacement.

orthopaedic bone cement (Palacos, Schering corp.) was used for fixation. Using a syringe, the cement was injected into the prepared medullary canals and the prosthesis was inserted into the cement, (fig. 2). Thirteen St. Georg prostheses (Waldemar Link) and one Steffee (DePuy) were inserted. On the left side the medial knee condyles were partly resected and the marrow cavities were opened through the defects.

Experiment III: The operative technique was the same as in Experiment II. The prostheses (St. Georg, Waldemar Link) were fixed in the medullary cavity on the right side with bone cement containing 0.5 g of gentamicin sulphate per 40 g of powder (Palacos R with Gentamicin Schering corp.) On the left side regular bone cement (Palacos R, Schering corp.) was used. An intramuscular injection of 150 mg of cephalothin sodium (Keflin^{R}, Lilly) was given prophylactically to each animal immediately before the operation to prevent peroperative infection.

Experiment IV: Operative technique as in Experiment II. All prostheses were fixed with regular bone cement (Palacos R, Schering corp.) in the right knee joints. Prophylactic antibiotics were used as in Experiment III.

Inoculation of bacteria and observation period

Experiment I: On the first postoperative day 1 ml of a suspension of *Staphylococcus aureus* (Wood 46) was injected into an auricular vein of seven rabbits. Simultanously seven other rabbits received injections of 0.5 ml of the same bacterial suspension injected into the postoperative hematoma. Daily injections were continued for three days. The experimental period was one week in the group receiving local inoculation and two weeks in the intravenously inoculated group.

Experiment II: Daily intravenous injections of a suspension of the experimental strain (*Staphylococcus aureus* Wood 46) was commenced on the first postoperative day. The numbers of injections given varied between two and five depending on the degree of impairment of the rabbits general condition. The animals were sacrificed after four weeks.

Experiment III: Postoperatively the rabbits were housed in individual cages for six to eight weeks before the bacterial injections were started. *Staphylococcus aureus* (Wood 46) was given intravenously once daily for one to three days depending on the general condition of the animals. The animals were sacrificed at 25 days after the inoculation.

Experiment IV: The bacterial inoculation was done at seven weeks postoperatively. Intravenous injections of a suspension of *Propionibacterium acnes* (ATCC 6919) were given once daily for three consecutive days. The animals were killed 46 to 48 days later.

Inoculation size and observation period are summarized in table 5 and fig. 4.

TABLE 5. Experimental data.

Experiment	Number of operated animals	Mean weight (±SD)	Time interval operation – inoculation	Time interval inoculation – sacrifice	Number of bacterial injections	Average number of bacteria inoculation/injection
I Direct inoculation	7	2730±250	1 day	9 days	3	3.2×10^9 S. aureus
I Intravenous inocualtion	7	2710±220	1 day	13 days	3	7.2×10^9 S. aureus
II Intravenous inocualtion	14	2770±210	1 day	4 weeks	1–5	3.6×10^9 S. aureus
III Intravenous inoculation	23	3100±275	6–8 weeks	4 weeks	1–3	1.2×10^9 S. aureus
IV Intravenous inoculation	10	2780±200	7 weeks	7 weeks	3	1×10^8 P. acnes

I - IV refers to Experiments listed on page 3.

Autopsy

In order to prevent contamination of culture specimens, sterile instruments were used for each location and each anatomical layer. The organs were opened and cultures were obtained by the swab technique using cotton tipped applicators. In addition to the surgical areas, cultures were taken from the lungs, the kidneys, the liver and the heart. The heart was taken out, burned over a flame and punctured for a blood culture specimen. Furthermore, tissue cultures were taken from all the operative areas.

Experiment I, II and III: Each specimen was cultured on a blood agar plate incubated aerobically and a hematin agar plate incubated in 10% CO_2 overnight at 37°C. If no growth was observed the plates were incubated for another 24 hours. Colonies suspected of being *staphylococci* were tested for desoxyribonuclease and coagulase (Dornbusch et al. 1976). Phage typing was done according to standardized methods (Blair and Williams 1961) using phages from the International reference laboratory in Colindale, England.

Experiment IV: The specimens were put into CO_2-filled sterile glass tubes and examined within one hour at the laboratory. Gram-stained smears were examined. Freshly prepared blood agar plates were inoculated and incubated anaerobically for seven days using the Gas-Pak system (BBL, Cockeysville, Md. USA). Aerobic cultures on blood agar plates were also made. Prereduced chopped meat broth was inoculated and incubated anaerobically. Subcultures were made on days four and seven on blood-agar plates incubated aerobically and anaerobically. The anaerobic bacteria were identified using morphology, by biochemical test, and gas-liquid chromatography (Holdeman et al. 1977).

Biotyping of *Propionibacterium acnes* isolates were carried out using fermentation of inositol, maltose, mannitol, and sorbitol (Pulverer and Ko 1973).

Serotyping of *Propionibacterium acnes* isolates was made as described by Voss (1970).

Phagetyping: Six *P.acnes* typing phages together with their propagating strains obtained from Dr Webster were used for phage typing (Webster and Cummins 1978).

Microradiography (Experiment II and IV) *and Histology* (Experiments II, III, and IV).

The distal parts of the femurs were split in the sagittal plane through the prostheses (fig. 3). One part was embedded in methylmethacrylate from which microradiograms were obtained from 50-100 µ ground sections. The other part was decalcified, embedded in paraffin, and sections stained with hematoxylin and eosin were prepared.

Serology (Experiment IV).

Blood samples were drawn from an auricular vein before the operation, one week before the bacterial injections and at sacrifice. Sera were analysed by the agglutination technique to the antigen, prepared as

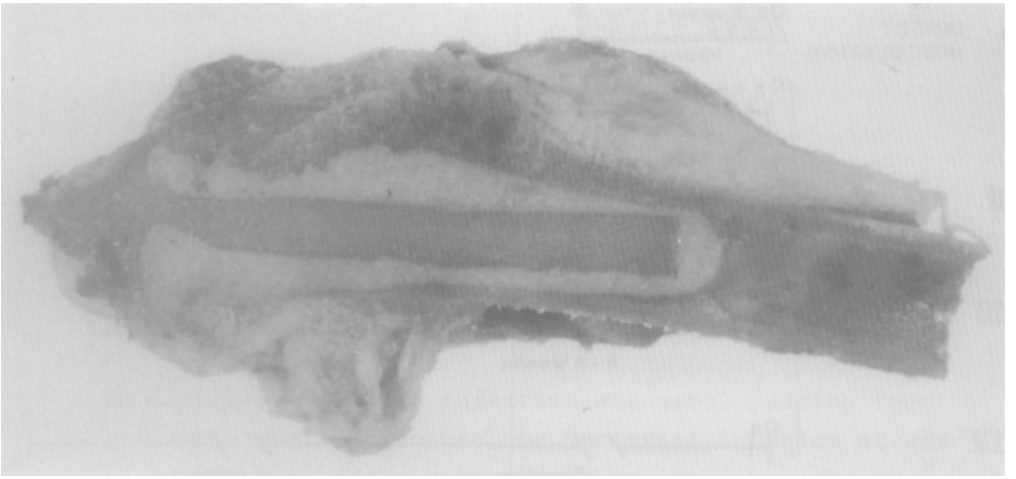

Fig. 3. The distal femur sectioned in the sagittal plane through the prosthesis, Experiment II (see list on page 3).

described by Holmberg et al. (1975), and carried out as a conventional Widal test, and by immunoelectroosmophoresis (Wadström et al. 1974).

Scintigraphy (Experiment IV)

All operated rabbits were examined at one week before the bacterial challenge and one week before sacrifice by ^{99m}Tc-methylenediphosphonate and ^{67}Ga-citrate bone scans.

The experimental phases are schematically illustrated in fig. 4.

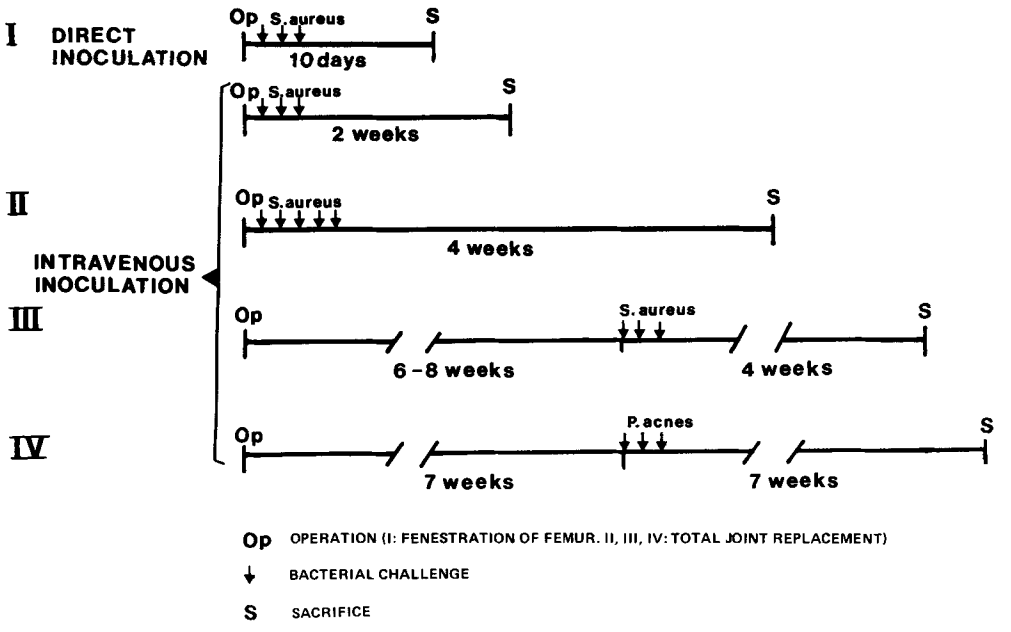

Fig. 4. Schematic illustration of Experiments I - IV listed on page 3.

RESULTS

No peroperative wound infection was seen. Judging from the rabbits that received the bacterial inoculum at six to eight weeks after surgery the operative procedure *per se* caused a weight loss of the animals. However, at the time of initiating the bacterial injections they had regained their initial body weight (Experiment III, IV). Postoperatively the rabbits moved freely in the cages although the function of the leg (or legs) with the joint replacement was affected to some degree. After replacement of both knee joints in a rabbit considerable stresses are working on the implant which in Experiment III was reflected by the high degree of loosening found in 16/23 on the right side and in 15/23 on the left side.

The majority of the rabbits in the experimental groups challenged with *Staphylococcus aureus* were severely affected by the bacteremia. This was reflected by a high death rate following the bacterial injections. Eight rabbits died close to the first inoculation, probably as a result of bacterial toxins. During the first week after the bacterial challenge another 11 rabbits died apparently following a manifest septicemia with the experimental strain of *Staphylococcus aureus* In one case dissemination with *Escherichia coli* had occurred.

In the group of rabbits injected with *Propionibacterium acnes* (IV) no reactions or weight loss were noticed after the bacterial injections.

Bacteriology

Experiment I: In the seven rabbits that had been locally inoculated at the femur defect,cultures from three defects with and three without cement yielded *S. aureus* (Wood 46) at the end of the observation period. Two intravenously inoculated rabbits died untimely. Only two of the operated femurs were found to be sterile, while four cemented and four uncemented femur defects showed growth of *S. aureus* The bacterial evaluation showed that at the end of the experiment bacteremia had disappeared in all the intravenously infected animals. *S. aureus* was, however, frequently isolated from the kidneys. (Table 6).

TABLE 6. Results of the bacteriologic analysis in Experiment I (see list on page 3).

	Rabbit No.	Heart Blood	Kidney	Lung	Liver	Right Femur (cement)	Left Femur
Animals receiving direct inoculation	1	-	-	-	-	-	-
	2	-	-	-	-	-	S.a.
	3	-	S.a.	-	-	-	-
	4	-	-	-	-	S.a.	-
	5	-	-	-	-	-	S.a.
	6	-	-	-	-	S.a.	S.a.
	7	-	-	-	-	S.a.	-
Animals receiving intravenous inoculation	1	-	S.a.	G-rod	-	-	S.a.
	2	-	S.a.	-	-	S.a.	S.a.
	3*	E.coli	E.coli S.a.	E.coli	E.coli	E.coli	E.coli
	4	-	S.a.	-	S.a.	S.a.	S.a.
	5*	S.a.	S.a.	S.a.	S.a.	S.a.	S.a.
	6	-	S.a.	-	-	S.a	-
	7	-	-	E.coli	-	S.a.	S.a.

S.a. = *Staphylococcus aureus* of the experimental strain (Wood 46) proved by phage typing.

* Died before the end of the experiment.

Experiment II: The macroscopic findings from the prosthetic joints revealed obvious infection with pus in three cases but the high rate of loosening (5/8 infected joints) may also be indicative of infection. The sham operated control knee joints yielded growth of the experimental strain in 3/4 cases after one week but were negative (1/10 cases) after four weeks. Cultures from all four endoprostheses yielded growth of *S. aureus* after one week. At the end of the four weeks experimental period 8/10 endoprostheses had positive cultures. In one case there was growth of *enterococci* while in the remaining seven joints cultures were positive for *S. aureus* (Wood 46). Although macroscopic signs of infection were lacking in the kidneys, *S. aureus* colonization was noted in four cases. Urine culture at 10 days before sacrifice showed growth of *S.aureus* in five cases. There was no correlation between positive culture from urine, kidney and prosthetic knee joint. All cultures other than those mentioned above were negative at four weeks after the operation (table 7, fig. 5).

TABLE 7. The distribution of positive cultures with *Staphylococcus aureus* (Wood 46) following intravenous inoculation in Experiment II (see list on page 3).

Specimen	4 rabbits spontaneously dead 1 week	10 rabbits killed after 4 weeks
lungs	0	0
blood (heart)	0	0
r + l kidney	3	4
liver	1	0**
spleen	2	0
humerus	2	0
prosthetic knee	4*	7***
sham operated knee	3	0

* Both the experimental *Staphylococcus aureus* strain and *E.coli* in one case.

** *E.coli* in one case.

*** *Enterococci* in one additional case. The total number of infected prosthetic knees thus = 8.

Experiment III: 25 days after the bacterial challenge four rabbits gave positive cultures with *S. aureus* from the area of the total joint prostheses and in three rabbits both knees were infected (table 8). Obviously there was no difference in the incidence of infection of the TJR whether or not gentamicin had been used in the bone cement. An almost complete dissemination of the experimental *S. aureus* strain occurred in the early deaths. The subsequent time groups revealed a decreasing degree of visceral contamination. (Table 8, fig. 5).

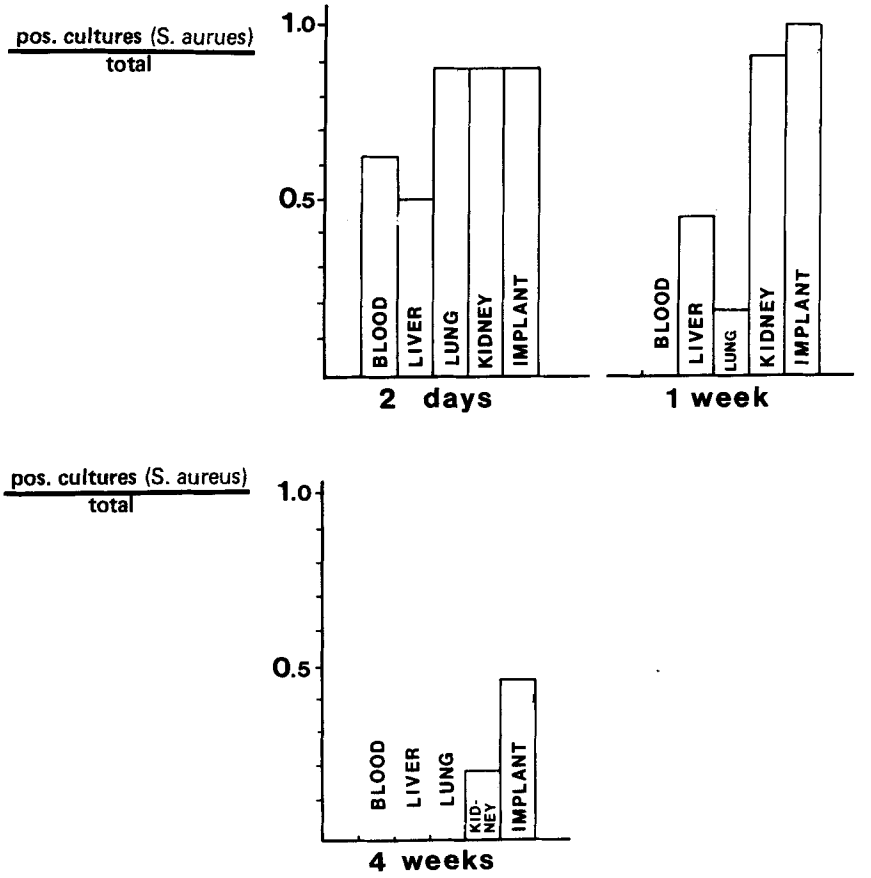

Fig. 5. The distribution of infections in Experiments II and III (see list on page 3) 2 days, 1 week and 4 weeks after intravenous inoculation of *Staphylococcus aureus* (Wood 46).

TABLE 8. Bacteriological results in rabbits intravenously injected with *Staphylococcus aureus* (Wood 46) at six to eight weeks following total joint replacements in both knees (Experiment III, se list on page 3).

	Positive cultures with experimental strain/total		
Period of infection	< 2 days	6 days	25 days
lung	7/8	2/5	0/10
blood (heart)	5/8	0/5	0/10
kidney	7/8	5/5	0/10
liver	4/8	3/5	0/10
right knee (+Gentamicin)	7/8	5/5	3/10*
left knee	7/8	5/5	4/10*

* Three rabbits had pos. culture from both knees.

The continued antibacterial activity of the gentamicin cement implant at the end of the experiment was *in vitro* verified by an agar-diffusion test. Small pieces of the cement implant were put on agar plates containing Antibiotic Medium No 2 (Difco) pH 7.9 and inoculated with *Bacillus subtilis*. After incubation at 37°C overnight, inhibition of the bacterial growth around the pieces was observed and interpreteded as gentamicin activity. No quantitative study was made. Pieces of the ordinary bone cement free from antibiotics did not show any inhibition zones. The findings of pus in two joints with gentamicin cement which yielded negative cultures was the only sign that might indicate antibacterial activity of the implant *in vivo*.

Experiment IV: All blood and visceral cultures were negative except in one rabbit, in which *P. acnes* (ATCC 6919) was cultured from the right kidney. Growth of the experimental strain of *P. acnes* occurred in five total joint replacement. The growth was restricted to the cement-bone area, except in one prosthetic joint, in which, *P. acnes* grew in a biopsy specimen from the capsular tissue of the joint. The unoperated left knee joints as well as the control animals were all negative. (Table 9).

TABLE 9. Results of Experiment IV (see list on page 3) in which *Propionibacterium acnes* (ATCC 6919) was intravenously injected seven weeks after implantation of total joint replacements. Analyses performed seven weeks after the bacterial inoculation.

	BACTERIAL CULTURES			ANTIBODY TITER AT SACRIFICE		SCINTIGRAPHIC RESULTS	
Rabbit number	Prosthetic area	Blood and viscera	Unoperated knee joint	Agglutination	Osmoelectrophoresis	^{99m}Tc–MDP	^{67}Ga–citrate
1	–	–	–	–	–	+	–
2	P.acnes	–	–	2+	1+	+	+
3	–	–	–	1+	–	+	–
4	–	–	–	–	–	+	–
5	–	–	–	1+	–	+	–
6	P.acnes	–	–	2+	1+	+	+
8	–	–	–	–	–	+	–
9	P.acnes	–	–	–	–	+	+
10	P.acnes	P.acnes (kidney)	–	–	–	+	–
11	P.acnes	–	–	2+	1+	+	–
12*		–	–	–	–		
13*		–	–	–	–		
14*		–	–	–	–		

* unoperated control animals
P.acnes the experimental strain of Propionibacterium acnes
\+ increased isotope activity
1+ immune reaction against P. acnes (2+ strong reaction)

Microradiography

Experiment II: Four weeks after operation and bacterial challenge with *S. aureus* the examination revealed signs of resorption cavities and periosteal new bone formation in all the infected cases. Endosteal new bone formation, mostly at the tip of the bone cement, was evident in 6/8 infected cases. In the sterile joints both resorption cavities and endosteal new bone formation occurred in one while neither showed periosteal new bone formation.

Experiment IV: Specimens from five rabbits were examined. Only one of them showed no growth of *P. acnes* at the prosthesis. There were no obvious signs of osteolysis or periosteal new bone formation. A thin calcified layer around the cement was usually seen.

Histology

After four weeks the histological analysis in the experiments concerning *S. aureus* (II and III) showed in all cases an inflammatory response of varying degree without clear correlation to infection. Abscess formation and areas of focal bone necrosis were, however, apparent in the infected cases. In Experiment IV (with *P. acnes* as the infecting agent) the inflammatory response was comparatively much less pronounced and of chronic type consisting mostly of lymphocytes, histiocytes, and some giant cells. Abscess formation and areas of focal bone necrosis were lacking. Generally the bone cement was more or less surrounded by a thin membrane consisting of fibroblasts and inflammatory cells. In some cases small areas of new-bone formation could be seen in the membrane and sometimes it was more pronounced and organized at the tip of the cement inside the medullary cavity. In most cases the innermost layer of the cortical bone, close to the cement or membrane, seemed dead with empty osteocyte lacunae.

Serology

Experiment IV: No antibodies against *P. acnes* were detected in sera obtained before the operation and the bacterial injections. At autopsy, seven weeks after the injections of *P. acnes*, antibodies were detected by using the agglutination technique in sera from five rabbits. Strong immune reactions were correlated to growth of *P.acnes* in the prosthetic area of three rabbits. Sera from these three animals also reacted against the corresponding antigen from *P.acnes* when using immunoelectroosmophoresis. As a comparison, sera from three unoperated rabbits were tested against *P. acnes* No antibodies were detected in sera from these animals. (Table 9).

Scintigraphy

Experiment IV: Bone images with ^{99m}Tc-MDP showed increased radionuclide activity corresponding to the bone surrounding the total joint prostheses in all cases. The activity was not changed between the examinations done one week before and six weeks after the bacterial inoculations.

With ^{67}Ga-citrate the prosthetic joint of five animals had a slightly increased radionuclide activity one week before the bacterial injections. Six weeks after the period of bacteremia three prosthetic joints showed a significant progression of increased activity. The remaining images had unchanged activity. All three cases with increased isotope activity had bacterial growth at the prostheses. (Table 9).

GENERAL DISCUSSION

The experimental model

The importance of hematogenous dissemination in osteomyelitis is well known (Trueta 1959, Winters and Cahen 1960, Gerszten et al. 1970). In experimental studies of hematogenous infections great care must be taken that the spread of bacteria and the resistance to infection take place under similar conditions in the experimental animal as it does in man. In this respect there are no principal differences between mammals. The cardiovascular system of the rabbit is similar to that of man. Also the rabbits resistance to infection is considerable (Wadström 1981). The establishment of bone infection by experimental means has been found uncertain, unless the normal host defences are modified. Norden (1970) found that a combination of bacteria and a sclerosing agent was required to induce osteomyelitis in the rabbit tibia. He injected sodium morrhuate followed by a suspension containing $3x10^6$ *Staphylococcus aureus* directly into the medullary cavity. Likewise, van Wingerden et al. (1974) also used sodium morrhuate in a study of *Pseudomonas osteomyelitis* in rabbits and reported that infection did not persist if a culture was injected without the sclerosing agent. Andriole et al. (1973) found that the dose of *staphylococci* which was unable to infect the normal rabbit tibia was capable of producing chronic osteomyelitis in the presence of an intramedullary nail.

The development of a hematogenous osteomyelitis is dependent on several factors, the most important of which are probably, the individual's resistance to infection, the concentration of bacteria in the blood, and the virulence of the bacteria. The majority of hematogenous bone infections involve pathogenic bacteria, especially *S. aureus* (Winters and Cahen 1960, Gerszten et al. 1970) but anaerobic bacteria have also been reported (Raff and Melo 1978). Probably, a certain blood concentration of

bacteria must be reached to result in an osteomyelitic process. With lowered resistance to infection the necessary concentration is probably less. A large number of bacteria have been inoculated in the present experiments and probably resulted in bacterial concentrations exceeding what is usual in clinical bacteremia. The relatively high frequency of metastatic infection reported in our material must be regarded in relation to the size of the inoculation and thus cannot be regarded as representative of the clinical situation. Inoculation with *S. aureus* resulted in significant general toxicity in the animals which was not correlated to the size of the inoculation but rather to the virulence of the bacteria and their predisposition to cause fulminent septicemia. The infections that were noted at the end of the experiments were most likely caused by a transient bacteremia in the animals that survived. That such is the case is supported by Experiment IV where the animals remained asymptomatic despite repeated inoculations with *P. acnes*. Even though the bacteremia did not result in any general symptoms, the implants were found contaminated in half the rabbits. Thus the experimental model appears to be adequately designed for studies on the principles of bacterial dissemination in bacteremia.

If the preventive measures described in the introduction are observed in total joint replacement procedures, the postoperative infection rate ought to be around one per cent. This level, while low, must however be considered important because of the consequences an infected joint replacement implies. Added to this figure is the cumulative risk for hematogenous infection of the joint prosthesis during the remaining life span of the patient. Obviously the implantation of metal, polyethylene, and acrylic cement leads to alterations in the local biological environment surrounding the implant. The decreased resistance to infection has certainly a multifactorial background. Petty (1978) demonstrated *in vitro* the inhibitory effect of acrylic cement on the ability

of white blood cells to phagocytize bacteria. Also joint fluid containing metal debris from joint prostheses has been shown to influence the white blood cells (Cracchiolo and Revell 1980). The most important factor that predisposes to infection of implants was considered by Marks (1980) to be the avascularity of the bone.

A few experimental models for studying infection problems in TJR are reported in the literature. Elson et al. (1977) studied contamination of cement plugs implanted in the tibia of rats. Schurman et al. (1978) replaced the femoral condyles of rabbits with cement and a small metal cylinder to form a hemiarthroplasty. Marks (1980) cemented a steal rod in the proximal tibia of rabbits. In total joint replacement load bearing stresses on the prosthesis are transmitted to the bone through the cement. The cement is usually surrounded by a thin layer of fibrous tissue (Willert et al. 1974). The development of this membrane-like structure seemed to be the result of load bearing stresses transmitted to the bone-cement interface (Spinelli 1976). The cortical bone adjacent to the cement and fibrous tissue is to a varying degree necrotic, but it is thought to revitalize with time (Willert et al. 1974. Rhinelander et al. 1979). Because there is a space between the necrotic bone and the cement it is conceivable that movements between these structures can take place with load bearing. Such movements involve a risk of local trauma and could possibly contribute to the susceptibility of infection in that area. Willert et al. (1974) has shown that loosening of the implant caused hemorrhages and fibrous exudates within the fibrous membrane around the cement.

In experimental studies of the disposition for infection of TJR, analogous to the discussion above, load bearing should be applied to the implant, that is, a functional prosthesis should be aimed at. The experimental model used in Experiments II, III and IV were constructed to resemble TJR in patients. In this model the

functional condition has been achieved.

The histologic analysis of the tissues surrounding the experimental implant demonstrated striking similaraties to corresponding investigations in patients. The cement was, to a variable degree, surrounded by a layer of fibrous granulation tissue, which contained, besides fibroblasts, a variable number of inflammatory cells and occasionally giant cells. The cortical bone adjacent to this fibrous membrane or the implant was frequently devoid of osteocytes in the lacunae which we, like Rhinelander et al. (1979), interpreted as a sign of bone-necrosis. These findings are in good agreement with the reactive changes around THR as described by Willert et al. (1974) and Mirra et al (1976). Periosteal new bone formation as well as osteolytic areas around the cement were seen microradiographically to appear with *S. aureus* infected prostheses. These changes, too, are consistent with the clinical situation as it is radiologically revealed with periosteal reactions around the shaft of the femur and resorption of bone surrounding the cement (Bergström et al. 1974, Hunter and Dandy 1977).

Hematogenous dissemination of the operative wound and the cement implant

It seems probable that the TJR by modifying the local defence mechanisms creates a *"locus minoris resistentiae"* to infection. The importance of the bone cement in this connection has been discussed. That there is a zone of necrotic bone surrounding the cement has been established (Willert et al. 1974, Jefferis et al. 1975, Reckling and Dillon 1977. Linder 1977, Rhinelander et al. 1979). Marks (1980) studied the significance of different local circulating conditions in the development of infection after direct inoculation with *S. aureus* of cement implants in the proximal tibia of rabbits. He came to the conclusion that the most important intrinsic predisposing factor in deep sepsis following TJR was avascularity of the surrounding bone.

Hematogenous infection of plugs of cement in the rabbit tibia was studied by Elson et al. (1977). Bacteremia was obtained by intravenous inoculation of *S. aureus* administrered within half an hour after the termination of the operation. Two weeks after the inoculation 70 per cent of the implants were infected. If the bacteremia was delayed to six weeks after the operation the corresponding infection rate was 17 per cent. The authors were of the opinion that the relatively low infection rate obtained with the delayed bacteremia suggested that recently traumatized tissue played a part in rendering the area prone to infection. Unfortunately, control animals, operated on without the plugs of cement, were not included, and hence the significance of the cement itself is hard to evaluate.

Our intention in Experiment I was to study wound infection following bacteremia and to find out whether an implant of cement in the wound would further increase the risk for infection. The first inoculations were delayed until the day after operation in order to avoid contamination of the wound by an active hemorrhage. We found in our material that the presence of a cement implant did not change the infection rate of the wound whether the inoculation was local or intravenous. Like the study reported by Elson et al. (1977), bacteremia with *S. aureus* resulted in a high infection rate of the wound. The degree of bacteremia, that is, the concentration of bacteria in the blood, is probably of decisive importance in the dissemination of the microorganisms to different tissues. Brief bacteremic episodes may occur in association with daily activities such as tooth brushing and bowel movements (Everett and Hirschmann 1977). Bacteremia emanating from such activites is, probably of a low grade and insignificant as a source of hemtogenous infection. As mentioned in the introduction, a number of different procedures and manipulations of the patients carry a risk for symptomatic bacteremia. Endocarditis has been reported in association with bacteremia of this type

(Everett and Hirschmann 1977). Infection and especially trauma of infected tissues carry a higher risk of bacteremia (Sullivan et al. 1973). In such circumstances the risk of established metastatic infection might be great. If a transient bacteremia would happen to occur during surgical procedures there is a certain risk for peroperative hematogenous seeding of the wound tissues although the grade of contamination is probably low.

Experiment I shows that recently traumatized tissue is easily contaminated by *S.aureus* bacteremia. The cement implant is of no significance for the risk of contamination in the fresh wound. As pointed out in the introduction, the extraordinary disposition to infection of TJR is a general clinical experience. In Experiment II the observation period was prolonged to about four weeks. During this observation period contamination was completely eliminated in the sham operated joints while an infective process prevailed with the experimental bacteria in 7/10 joints with implants. This difference is probably not caused by a different dissemination of the bacteria but rather by the decreased defence against infection at the total joint implant.

Hematogenous dissemination of total joint replacements (Experiments II, III and IV)

Experimentally, bacteremia has been brought about by injecting bacteria subcutaneously (Krizek and Davis 1966) or intravenously (Howe 1968 and 1969). The intravenous administration has mostly been used in investigations of hematogenous infection (Thompson and Dubos 1938, Rigdon 1942, Howe 1968 och 1969, Elson et al. 1977). In the present experimental series the bacterial suspension was injected into an auricular vein. The defence mechanisms in the circulation, the reticuloendothelial system and the passage through the lungs ought to reduce substantially the number of bacteria which momentarily are spread to the tissues. From fig. 5, which summarizes the frequency of positive cultures in Experiments II and III,

it is evident that the number of *S. aureus* used here has brought on the possibility of bacterial contamination in most tissues The animals which died early, within two days and one week, respectively, after the bacterial injections, demonstrated a frequent contamination of the blood and investigated tissues. The elimination of bacteria appeared to take place primarily in the bloodstream and later on in the liver and lung tissues. The bacteria often remained in the kidneys during a long period. The latter finding has previously been reported by several authors (Thompson and Dubos 1938, Norden 1970, Elson et al. 1977) However, the renal tissue did not show any signs of macroscopic infection. Even more frequently than in the kidneys, the bacteria were demonstrated at the implants. It seems reasonable that dissemination of bacterial strains other than *S. aureus* would also occur in a similar way after intravenous administration (Howe 1968 and 1969). Though not common, osteomyelitis may follow anaerobic bacteremia (Raff and Melo 1978). As demonstrated by experiment IV, anaerobic bacteremia may also result in hematogenous dissemination to implants and kidneys. In that experiment no animals died following inoculation, so the dissemination to other organs cannot be stated. Thus, intravenous injection in the rabbit of both aerobic and anaerobic bacteria results in a bacteremia which involves a risk for metastatic foci in areas with reduced resistance to infection.

The high rate of contamination that was noted in Experiments II, III, and IV demonstrates that the altered biological environment surrounding a TJR involves a substantial risk of contamination. The bacterial strain is not considered to play a decisive part in the risk of contamination, as both *S. aureus* (III) and *P. acnes* (IV) caused comparable rates of contamination (35 and 50 per cent respectively). It appears then that any bacteria occurring in the bloodstream present essentially the same risk of contamination of a TJR. This risk seems to be more pronounced in connection with the operation than

in the subsequent healing periods. When the inoculation of *S. aureus* was made early in the postoperative period, the resulting infection rate was 70 per cent (7/10) while, if the inoculation was given in the sixth to eight postoperative week, the corresponding rate was 35 per cent (7/20). This difference could be explained by the increased risk of contamination of recently traumatized tissues. The sham operated joints in Experiment II showed a high contamination rate in connection with the inoculation. However, four weeks after the inoculation the contamination was completely eliminated. Obviously, there is a heavy resistance to infection in healthy tissues, whereas the altered biological environment of TJR implies a reduced defence resulting in high infection rates after contamination.

Peroperatively, the TJR is exposed to a significant risk of contamination. It is hardly surprising that, if prophylactic measures are not taken, the striking susceptibility of infection of the area leads to the high postoperative infection rates reported. To reveal the hematogenous infections in the group of early postoperative infections is difficult if there is not a bacteriological agreement with a primary foci of infection. There are no definite cases of early hematogenous infections reported in the literature except for a few cases associated with urinary tract infections (Irvine et al. 1974, Amstutz and Kass 1977). Undoubtely, the patients are peroperatively subjected to a risk of bacteremia by various manipulations and instrumentations. Probably most of these bacteremias are not of the magnitude required to involve a significant contamination risk of the wound tissue. Nevertheless, it seems motivated to avoid procedures that involve a risk of bacteremia, like the use of urinary catheters. Patients with an active infection, especially if the focus of infection is traumatized, are exposed to an increased risk and should not be subjected to TJR.

In the literature, infections of total joint arthroplasties appearing after the first three postoperative months are called late or delayed. As regards late infections, several authors, e.g. Charnley (1972 and 1979) and Müller (1974), consider that they are due mainly to peroperative contamination, whereas others, e.g. Buchholz (1973) stress the importance of the possibility of hematogenous spread. To my knowledge the only earlier experimental studies on late postoperative infections involving implants are those carried out by Elson et al. (1977) and Marks (1980). Marks showed that the incidence of infection remaining after direct inoculation of implants fell from 55 per cent to 15 per cent when the inoculation was delayed until the fourth postoperative week. Elson et al. found that the said incidence was 70 per cent and 17 per cent, respectively, when they postponed the intravenous inoculation until the sixth postoperative week. A similar study is in our material represented by Experiment III, in which a solution of *S.aureus* was injected intravenously in the sixth to eighth postoperative week. After an observation period of four weeks, cultures from seven (35 per cent) of 20 prostheses yielded growth of the injected bacterial strain. Allowing for differences in the design of the experiments, a comparison of our results with those reported by Elson et al. and Marks shows that the incidence of infection was much higher in our material. The stress of the implant can cause minor movements between the bone and the cement, thus subjecting the fibrous granulation tissue in the interface to tears with accompanying bleeding and exudation. Under such circumstances the risk of contamination in bacteremia is likely to increase. In cases of loosened prostheses Willert et al. (1974) observed, histologically, tears with hemorrhages and exudates in the connective- tissue membrane surrounding the cement. Analogously, loosening would predispose to hematogenous infection.

At re-operation of TJR an anaerobe bacterial flora has been demonstrated in about 25 per cent of cases (Nolan et

al. 1975, Buchholz et al. 1977, Carlsson et al. 1978, Blomgren et al. 1981). Anaerobe bacteria have a pattern of spread similar to that of aerobe bacteria in the environment of the operating theatre, thus constituting a peroperative contamination risk (Hambraues and Benediktsdottir 1980). In most papers dealing with anaerobic infections of total replacements the authors seem to regard the cases as delayed infections after peroperative contamination (Kamme et al. 1974, Nolan et al. 1975, Carlsson et al. 1978, Petrini et al. 1979). Buchholtz (1973), on the other hand, stressed the occurrence of anaerobic bacteremias and considered that the anaerobic infections were attributable to hematogenous spread.

Experiment IV show that anaerobic bacteria have the same pattern of spread to TJR as aerobic microorganisms. The experimental animals were given intravenous injections of *Propionibacterium acnes* in the seventh week after implantation of the prostheses. At seven weeks after the inoculation 50 per cent of the implants yielded growth of *P. acnes*. The bacterial growth was restricted to the parts of the prosthesis situated intramedullary, except in one case in which *P. acnes* grew also in a specimen from the joint capsule. This local restriction of bacterial growth supports the afore-discussed possibility not only of lowered resistance to infection but also of increased risk of contamination in the bone-cement interphase. Restriction of bacterial growth to this bone-cement area could also explain the frequent occurrence of negative cultures from joint aspirations in patients with bacterial growth from peroperative specimens at reoperation (Fremont-Smith 1974).

Serum antibodies against anaerobe bacteria isolated at reoperation of infected total arthroplasties have shown increased titres (Kamme et al. 1974. Petrini et al. 1979). The present study revealed serum antibodies against *P. acnes* in five rabbits but a positive correlation existed between strong immune reactions and findings of

bacterial growth in three joint replacements (table 9). Accordingly, serology might be of diagnostic aid in the evaluation of cases of unsuccessful arthroplasties.

Postoperatively, bone activity studied by ^{99m}Tc-MDP bone scans is increased and returns to normal in about six months (Williamson et al. 1979). An abnormal bone image is usually associated with significant disease but the differentiation between loosening and infection is very delicate (McInerney & Hyde 1978, Williamson et al. 1979, Weiss et al. 1979). Studies with ^{67}Ga-citrate, which has an affinity for leucocytes, could be helpful in the diagnosis of infection. In 1979, Reing et al. reported 19 positive gallium scans in 79 patients with painful TJRs. All the patients with positive gallium scans had positive cultures at the time of operation. Only one patient had a false-negative gallium scan. Unfortunately, Reing et al. did not specify the bacteriological findings at operation. In the present study the bone images with ^{67}Ga-citrate were positive in three instances. These were correlated to bacterial growth. Two false-negative scans were obtained. There were no histological alterations found that could explain the difference. Theoretically, virulent organisms, e.g. *S. aureus*, which cause significant acculumation of leucocytes, would be more likely to be reflected by positive gallium scans than anaerobe bacteria like *P. acnes*, the present experimental strain.

There were no macroscopic, microradiographic, or histologic signs of loosening of the prostheses in Experiment IV. Histologically, the cement was surrounded by a thin layer of fibrous granulation tissue with a cellular structure similar to that described by Willert et al. (1974) as a normal reaction to a cement implant. Evidently, the presence of *P. acnes* did not lead to any morphologic changes in the environment of the implant during the observation period. If it was capable of causing loosening of the prosthesis *P. acnes* would seem to require

an extended time period. The experimental results show that bacteremia due to *P.acnes* involved a great risk of contamination of TJR in our experimental model and thus support Buchholz' (1973) opinion that anaerobic infections after total joint replacements are due to hematogenous spread of the bacteria.

Gentamicin cement as a protection against hematogenous infection in the late postoperative period

Wahlig and Dingeldein (1980) carried out clinical and experimental long-term observations of antibiotics and bone cement and found that, both *in vitro* and *in vivo*, gentamicin was for a long period (at least five years) continuously released from the bone cement. The amount of released gentamicin decreased, however, quickly and after one week it reached only 1/100 of the values recorded *in vitro* during the first 24 hours. Their *in-vivo* experiments on dogs showed that concentrations of gentamicin remained in connective tissue and bone tissue close to the implants for as long as 22 months after the operation. The concentrations in the tissues fell quickly, however, and only in the first postoperative week did cortical bone contain gentamicin concentrations exceeding 4 µg/g of tissue. They were also able to demonstrate that, particularly in connective tissue and cancellous bone, highly varying concentrations of gentamicin (0 - 39 µg/g of tissue) remained for up to five years in specimens taken from tissue close to the implant in 18 patients. Residual gentamicin concentrations in the tissues might provide increased long-term postoperative local protection against infection, thus being effective in preventing late hematogenous infections. Elson et al. (1977) studied the incidence of infection of cement implants with and without gentamicin (0.5 g/40 g powder) both in early (1/2 hour) and in late (six weeks) postoperatively induced bacteremia with *S. aureus*. At early inoculation they obtained contamination of 42 of 60 cement implants without gentamicin and 2 of 42 implants with gentamicin. At late inoculation the corresponding figures were 11 of 64 and 1

of 12, respectively. According to the present author's calculations, the difference is statistically significant in the group in which the bacteria were injected at half--an-hour after the operation ($\chi c^2 = 40.24$), whereas in the group with late injection it is not significant ($\chi c^2 = 0.12$). Schurmann et al. (1978), using direct inoculation of *E. coli*, infected the knee joints of rabbits in which the femoral condyles had been replaced by bone cement and a metal cylinder. A gentamicin-dependent protection against infection could be demonstrated when the bacteria were injected immediately after the operation. When the inoculation was delayed for one week postoperatively, no conclusion could be drawn, since the control animals (with cement without gentamicin) did not become infected. Thus, the cited studies show a protective action of gentamicin cement against infection immediately after insertion but fail to demonstrate a lasting antibacterial effect *in vivo*. This view is supported by the results obtained in Experiment III. The experimental joints were implanted using ordinary bone cement on the left and gentamicin-containing bone cement on the right side. Intravenous inoculation of *S. aureus* was delayed for six weeks postoperatively. At four weeks after the bacterial challenge three rabbits had bilaterally infected implants, whereas six rabbits developed no infection at all. Further, in the group of rabbits examined on the sixth day after the bacterial challenge there was no inhibition of bacterial growth around the gentamicin-containing implant. The higher infection rate of our implants, compared with that found by Elson et al. (30 per cent as against 8 per cent), can again be explained by the loading stresses of the total joints in our experiments. The continued antibacterial activity of the whole implant of gentamicin cement at the end of the experiment was verifeid *in vitro* by an agar--diffusion test using *Bacillus subtilis*. The findings of pus in the two joints with gentamicin cement which yielded negative cultures was the only sign that might indicate antibacterial activity of the implant *in vivo*. The use of bone cement with gentamicin obviously does not

continue to release gentamicin in amounts sufficient to yield tissue concentrations that are high enough to prevent late hematogenous infection. The conclusion is that in order to protect patients with cemented endoprostheses from late hematogenous infection, bacteremia must be prevented.

GENERAL SUMMARY AND CONCLUSIONS

The dissemination of intravenously injected bacteria to surgical wounds and total joint replacements was studied in an animal model. Male New Zealand white rabbits were used as experimental animals and the operation was either a fenestration of the femur or a total joint replacement using human finger joint prostheses. The following conclusions were drawn:

1. Bacteremia during the immediate postoperative course involves a substantial risk of infection in the area of operation.
2. An acrylic cement implant does not influence the susceptibility to infection of the operation wound.
3. Hematogenous spread seems to be a source of infection as potent as local inoculation of bacteria.
4. Bacteremia often leads to a long-standing infection of the kidneys.
5. A joint implant has a high risk of becoming infected during bacteremia.
6. Bacteremia seems to involve a greater risk of infection in the early postoperative period than in the late period.
7. Gentamicin-impregnated bone cement does not protect a total joint replacement against late hematogenous infection.
8. *Staphylococcus aureus* (Wood 46) and *Propionibacterium acnes* (ATCC 6919) have the same ability to cause hematogenous infection of a total joint replacement.
9. Hematogenous infection with *Staphylococcus aureus* (Wood 46) of a total joint replacement results in alterations typical of acute bone infection, such as pus formation, bone resorption, and periosteal reactions.
10. Hematogenous infection with *Propionibacterium acnes* (ATCC 6919) of a total joint replacement did not result in any macro- or microscopic signs of infection.
11. Gallium bone scans and serological examinations may contribute to the diagnosis of total joint replacement infected with *Propionibacterium acnes* (ATCC 6919) when sterile specimens of aspirated joint fluid are obtained.

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