LATE ANAEROBIC HAEMATOGENOUS INFECTION OF EXPERIMENTAL TOTAL JOINT REPLACEMENT

A STUDY IN THE RABBIT USING PROPIONIBACTERIUM ACNES

G. BLOMGREN, H. LUNDQUIST, C.-E. NORD, U. LINDGREN

From the Karolinska Institute, Huddinge University Hospital, Stockholm

Haematogenous contamination of cemented total knee replacements with an anaerobic microorganism, Propionibacterium acnes, was studied in rabbits. Seven weeks after operation the bacteria were injected intravenously and seven weeks after this bacterial challenge the experimental bacterial strain was cultured from 50 per cent of the artificial joints. Bacterial growth at the prosthesis was not correlated to loosening. The results illustrate that anaerobic Gram-positive bacteria are also important in haematogenous infection of the total joint replacement.

Total joint replacement is well known to involve a high risk of infection. During the last decade the infection rate has been reduced by a number of preventive measures. These include the prophylactic use of antibiotics, either systemically or mixed in the bone cement, and improvements in hygiene, including the use of clean-air operation theatres. These precautions have reduced the incidence of infection after operation to about one per cent (Nelson 1977; Buchholz 1979). Infections with aerobic bacteria, such as Staphylococcus aureus, in the area around an orthopaedic implant result in loosening. Late infections in many cases are probably spread in the blood stream. During the last years a number of such cases have been reported (Ahlberg, Carlsson and Lindberg 1978). The incidence has been estimated by Charmley (1972) and Ahlberg et al. (1978) as approximately 0.3 per cent. In earlier experimental studies we have found that joint endoprostheses are extremely susceptible to haematogenous infection by Staphylococcus aureus both shortly after the operation and later (Blomgren and Lindgren 1980, 1981).

A considerable number of infections around prostheses are caused by bacteria with low virulence. Staphylococcus epidermidis was identified in 21 per cent of 392 infections of hip arthroplasties reported in a review article by Nelson (1977). The association between late loosening of the prostheses and the growth of anaerobic bacteria was noticed by Kamme et al. (1974) who found anaerobic bacteria in four out of seven revisions for total hip replacements which had failed. An incidence of around 20 per cent was found by Nolan et al. (1975), Buchholz et al. (1977) and by Carlsson, Josefsson and Lindberg (1978). In 1979 Petrini, Welin-Berger and Nord also reported 12 prostheses infected with anaerobic bacteria. Peptococci, Peptostreptococci and Propionibacterium acnes have been isolated in most cases (Kamme et al. 1974; Carlsson et al. 1978; Petrini et al. 1979). The possible method of entrance seems to be either by direct contamination during operation or by the blood stream. Contamination during operation would imply a latent period, sometimes of several years, before clinical symptoms appear.

Transient, usually asymptomatic, episodes of bacteraemia have been reported to occur in patients in connection with various kinds of manipulation and instrumentation, especially trauma of mucous membranes which has been shown to cause a high frequency of bacteraemia (Everett and Hirschmann 1977). In some hospitals between 6 and 20 per cent of the total number of positive blood cultures have yielded growth of anaerobic bacteria (Wilson et al. 1972; Williams et al. 1976; Noone, Abeyesundere and Bradley 1978). The anaerobic bacteria are known to cause endocarditis (Felner 1974); the incidence of Propionibacterium acnes seems to be high in this disease after open heart surgery (Levin 1966; Johnson et al. 1968).

Since total joint replacements create areas of low resistance to infection the possibility for a haematogenous anaerobic infection seems to exist. To study the risk of infection in the areas around joint endoprostheses by anaerobic bacteria occurring in the blood at a

G. Blomgren, MD, Orthopaedic Surgeon, Department of Orthopaedic Surgery
H. Lundquist, MD, Radiologist, Department of Diagnostic Radiology
C.-E. Nord, DD, Professor, Department of Oral Microbiology
U. Lindgren, MD, Associate Professor, Department of Orthopaedic Surgery

Requests for reprints should be sent to Dr G. Blomgren.

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late stage after operation, the present experiment was performed using an earlier model for studies of the haematogenous spread of *Staphylococcus aureus* (Blomgren and Lindgren 1980, 1981).

**MATERIAL AND METHODS**

In this study 14 male New Zealand rabbits with a mean weight of 2822 grams ± 206 were used. A total knee replacement was done on the right side in 10 rabbits. An intravenous inoculation of *Propionibacterium acnes* (10⁶ colony-forming units per millilitre) was given after a healing period of 46 to 48 days. One millilitre of the bacterial suspension was injected into the auricular vein once a day for three days. The control animals received the bacteria but were not operated upon. The animals were killed seven weeks after bacterial challenge. All experimental rabbits had neurolept analgesia with fentanyl-fluanisone administered intramuscularly in a dose of 0.5 millilitres per kilogram body-weight.

**Operation.** The operation was performed in a modern operating theatre and with disposable sterile dressings, including sterile adhesive drapes and a newly sterilised set of instruments were used for each rabbit. The knee was opened through a medial parapatellar incision and the patella dislocated laterally. The joint surfaces were resected and the medullary cavities were opened. Commercial orthopaedic bone cement (Palacos), was used for the fixation of the total knee prosthesis. This cement was injected into the prepared medullary canals with a syringe and the prosthesis was inserted into the cement. We used endoprostheses designed for replacement of human finger joints (St George Fingermittellgelenk endoprostheses), with one polyethylene and one cobalt–chrome–molybdenum alloy component, but without the hinge (Fig. 1). The wound was closed with polyglycolic acid sutures. An intramuscular injection of 50 milligrams of cephalothin sodium per kilogram body-weight was given to each animal immediately before the operation in order to prevent infection during the operation. The animals were then housed in individual cages before the injections of bacteria were started.

![Radiograph of the total knee replacement in a rabbit one week after implantation.](image)

**Serology.** *Propionibacterium acnes* (ATCC 6919) was grown to a density of 0.5 grams per litre in a peptone-yeast extract and glucose broth which was preduced and anaerobically sterilised (Holdeman, Cato and Moore 1977). It was then harvested by centrifugation, washed in saline, and stored at minus 20 degrees Celsius until used. The antigens associated with the cells were prepared as described by Holmberg, Nord and Wadstrom (1975). The agglutination technique of the sera from the rabbits to the prepared antigen was carried out as a conventional Widal test.

Immuno-electro-osmophoresis was carried out in 0.85 per cent (weight: volume) Nobel agar on glass slides 20 by 10 centimetres in a layer one millimetre thick. The buffer in the gels and the electrode vessels was 0.05 molar sodium phosphate buffer (pH 7.0).

The antigens (25 microlitres) were assayed in ten-fold dilutions and the sera from the rabbits (25 microlitres) in two-fold dilutions. The electrophoresis was carried out on LKB Multiphor apparatus. After electrophoresis at 15 volts for 60 minutes the plate was examined for precipitation lines and re-examined after staining with Coomassie brilliant blue R250 (Wadstrom et al. 1974).

The blood samples needed were drawn from an auricular vein before operation, at one week before the bacterial injections, and at the time of killing.

**Radiology and scintigraphy.** Three radiographs were taken for each operated rabbit: during the first week after operation; one week before the bacterial challenge; and at one week before killing. Only lateral projections with standard skeletal exposures were obtained.

All operated rabbits were examined by both technetium and gallium bone scans one week before the bacterial challenge and one week before killing. Ten megabecquerels of methylene diphosphonate labelled with technetium-99m and 7.5 megabecquerels of gallium-67 citrate were given intravenously to each animal. Bone images were obtained at three and 48 hours respectively after administration using a gamma camera with a collimator consisting of 4000 holes. Scintigraphy was carried out with 100,000 counts for the technetium-99m methylene diphosphonate and 50,000 counts for the gallium-67 citrate. The results were interpreted by one of us (HL) without knowing the results of the bacteriological examination.

**Necropsy and bacteriological examinations.** Necropsy was carried out 46 to 48 days after the bacterial challenge according to a detailed scheme. Using a new set of sterile instruments for each anatomical layer and each location, the organ was approached, opened, and a bacteriological specimen was obtained by a swab or tissue biopsy. Samples were taken from the cement-bone area of the prosthesis, the knee, the lungs, the kidneys, the liver, and the heart. The latter was taken out, burned over a flame and then punctured for a blood-culture specimen. The specimens were put into carbon dioxide-filled sterile glass tubes and examined within one hour in the laboratory. Freshly prepared blood-agar plates were inoculated and incubated anaerobically for seven days using the Gas-Pak system. Aerobic cultures on blood-agar plates were also made. Prereduced chopped-meat broth was inoculated and incubated anaerobically. Subcultures were made on the fourth and seventh days on blood-agar plates incubated aerobically and anaerobically. The anaerobic bacteria were identified morphologically, by biochemical tests and gas-liquid chromatography (Holdeman et al. 1977).

Biotyping of the *Propionibacterium acnes* isolates was carried out using the fermentation of inositol, maltose, mannitol and sorbitol (Pulverer and Ko 1973). Serotyping of these isolates was also made as described by Voss (1970). Six *Propionibacterium acnes* phages together with their propagating strains obtained from Dr Webster were used for phage typing (Webster and Cummins 1978).

**Preparation of microradiographical and histological slides.** The distal parts of the femora from five animals were carefully preserved and then split along the sagittal plane through the prosthesis. One half was embedded in methyl methacrylate and sections 50 to 100 microns thick used for microradiography. The other half was decalcified, embedded in paraffin, and sections were prepared and stained with haematoxylin and eosin.

**RESULTS**

One rabbit (Number 7) died during the operation and was not included in the study. All operative wounds healed without signs of infection. The general condition of the animals was moderately affected after operation.
resulting in a mean loss in weight of approximately 10 per cent. However, by the time the bacterial injections were given they had regained their previous body-weight. All the rabbits moved freely in the cages walking on the operated leg, although a slight degree of dysfunction was noticed. The rabbits did not react to the bacterial injections and their body-weight continued to increase. All the rabbits were killed seven weeks after the initial bacterial injection.

Necropsy. All the operated rabbits had a thickened capsule at the prosthetic joint. There was no case of purulent exudate or formation of abscesses. All the prostheses were firmly fixed to cement and bone and there were no macroscopic alterations of the viscera examined.

Bacterial cultures. All the blood and visceral cultures were negative apart from one rabbit (Number 10) in which Propionibacterium acnes was cultured from the right kidney. There was no positive culture from the unoperated left knee. Cultures of specimens taken from the cement-bone area in five rabbits yielded growth of Propionibacterium acnes (Table 1). One of these (Number 2) had additional growth in the capsular tissue of the joint. The bacterial isolates were identified as the experimental strain of Propionibacterium acnes by using biotyping (Type G), serotyping (Type I) and phage-typing (Type 1a). All the control animals (Numbers 12, 13 and 14) had negative cultures in the viscera and in the knees.

Serology. No antibodies against Propionibacterium acnes were detected in sera obtained before the operation or before the bacterial injections. At necropsy, seven weeks after the injections of bacteria, antibodies were detected in the sera from five rabbits using the agglutination technique (Table 1). Strong immune reactions were correlated with the growth of Propionibacterium acnes in the prosthetic area of three rabbits (Numbers 2, 6 and 11). Sera from these three animals also reacted against the corresponding antigen from Propionibacterium acnes when using immunoelectro-osmophoresis. As a comparison, sera from the three unoperated rabbits were tested but no antibodies were detected.

Radiology. In the sixth week after the bacterial challenge, a thin radiolucent zone around the cement of the femoral prosthesis was found in one of the rabbits (Number 3). None of the other examinations showed any signs of loosening or infection (Fig. 1).

Scintigraphy. Bone images with technetium-99m methylene diphosphonate showed increased radionuclide activity corresponding to the bone surrounding the total joint prostheses in all cases. The activity was not changed between the examinations made one week before and six weeks after the injections of bacteria. With gallium-67 labelled citrate the prosthetic joint of five animals had a slightly increased radionuclide activity one week before the bacterial injections. Six weeks after injection of bacteria three prosthetic joints

Table I. Results of the analyses performed seven weeks after injection of the rabbits with Propionibacterium acnes

<table>
<thead>
<tr>
<th>Rabbit number</th>
<th>Prosthetic area</th>
<th>Blood and viscera</th>
<th>Unoperated knee</th>
<th>Antibody titre at death</th>
<th>Scintigraphic results</th>
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<tbody>
<tr>
<td></td>
<td>Bacterial cultures</td>
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<td>Agglutination</td>
<td>Electro-osmophoresis</td>
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</table>

* unoperated control animals
P. acnes is the experimental strain of Propionibacterium acnes
1+ indicates an immune reaction against P. acnes (2+ is a strong reaction)
+ indicates increased isotope
MDP, methylene diphosphonate
showed a significant progression of increased activity (Figs 2 and 3). The remaining images had unchanged activity. All three rabbits with increased isotope activity (Numbers 2, 6 and 9) had bacterial growth at the prostheses.

**Microradiography and histology.** Specimens from five rabbits (Numbers 6, 8, 9, 10, 11) were examined. In one of these (Number 8) there was no growth of the experimental bacteria at the prosthesis. There was no obvious sign of osteolysis or periosteal new-bone formation on the microradiographs. The histological parts of the prostheses but in one rabbit additional growth was obtained from a specimen of the joint capsule. This suggests a decrease in the defence mechanisms of the tissues surrounding the cemented parts of the total joint prostheses. The occurrence of necrosis of the bone adjacent to the acrylic cement after operation which has been shown both experimentally (Linder 1977; Rhinelander et al. 1979; Blomgren and Lindgren 1980) and clinically (Willert, Ludwig and Semlitsch 1974), could partly explain the local decreased resistance and the possibility of bacterial growth. The restriction of bacterial growth to this cement-bone area could also explain the negative cultures from aspirations of the joint at reoperation in patients with bacterial growth.

Serum antibodies to the anaerobic bacteria isolated at reoperation of infected total joint arthroplasties have shown increased titres in humans (Kamme et al. 1974; Petrini et al. 1979). Our study revealed serum antibodies against *Propionibacterium acnes* in five rabbits and a positive correlation between the immune reactions and bacterial growth in three joint replacements. Serology therefore seems to be of diagnostic help in the evaluation of patients with failed arthroplasties.

After operation the technetium bone scans showed an increase in activity of the bone; this returns to normal after about six months (Williamson et al. 1979). An abnormal bone scan is usually associated with disease but the difference between loosening and infection is very slight (McInerney and Hyde 1978; Weis et al. 1979; Williamson et al. 1979). Scans using gallium-67 citrate, which has an affinity for leucocytes, could be helpful in the diagnosis of infection. Reing, Richin and Kenmore (1979) reported 19 positive gallium scans in 79 patients with painful total joint replacements. All the patients with positive gallium scans had positive cultures at the time of operation. In the present study the bone images with gallium-67 citrate were positive in three rabbits; these were correlated to bacterial growth. Two false-negative scans were obtained. There were no histological alterations found to explain the difference. Theoretically virulent microorganisms, like *Staphylococcus aureus* which cause significant accumulation of leucocytes, are more likely to be detected by positive gallium scans. In the present experiment the histological specimens showed rather weak signs of chronic inflammation. There was no case of established prostatic loosening macroscopically or by histology, radiology, or microradiography. One rabbit (Number 3), with negative cultures, at the time of death had acquired a thin radiolucent zone around the femoral cement, but this was not reflected by any obvious loosening. The presence of *Propionibacterium acnes* did not cause obvious reactions in the areas surrounding the prostheses during the experimental period. Fibrous granulation tissue with a cellular composition of a chronic inflammatory type and with foreign-body giant cells has

- **Fig. 2**
- **Fig. 3**

Gallium bone scans for rabbit Number 6 obtained before (Fig. 2) and six weeks after (Fig. 3) intravenous inoculation with *Propionibacterium acnes* after total replacement of the right knee. Increased isotope activity can be seen in the prothetic joint (arrow) associated with bacterial growth.

analysis showed fibrous granulation tissue in the area of the femoral condyles and around the bone cement. The tissue around the bone cement consisted of fibroblasts and inflammatory cells, mostly lymphocytes and histiocytes, and some giant cells. There were no abscesses formed or areas of bone necrosis except for empty osteocyte lacunae in the innermost layer of cortical bone in two rabbits (Numbers 8 and 9).

**DISCUSSION**

The experimental animal model was designed to resemble a human total joint replacement. This model was earlier used by us in experiments concerning the haematogenous spread of *Staphylococcus aureus* (Blomgren and Lindgren 1980, 1981). These experiments underlined the importance of the haematogenous origin of aerobic organisms early as well as late after total joint replacement. Our present study proves that anaerobic bacteria can also contaminate the area of total joint implants by haematogenous spread. The animals were therefore challenged with *Propionibacterium acnes* in the seventh week after the implantation of the prostheses, by which time all the rabbits had functional prothetic knees. The intravenous injections of bacteria did not result in any obvious change of the general conditions of the animals. Seven weeks later the bacterial growth was restricted to the intramedullary
been reported by Willert et al. (1974) as a normal reaction of bone cement and has also been found by us in specimens obtained at revision operations on both sterile and anaerobically infected patients. It appears that if Propionibacterium acnes is capable of causing loosening of the prosthesis then an extended period of time is required.

In conclusion anaerobic Gram-positive bacteria, such as Propionibacterium acnes, appear to have the same capability as aerobic Gram-positive bacteria, such as Staphylococcus aureus, to contaminate total joint replacements haematogenously in this animal model. If the results are valid in humans, patients with cemented endoprostheses would also be exposed to a certain risk of haematogenous infection during anaerobic bacteremia.

REFERENCES


