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Rabbit model of septic arthritis

Eighty-five rabbits were injected in one knee with *Staphlycoccus aureus* in order to study the time-related changes in untreated septic arthritis up to 3 months. In the synovial membrane a severe release of lysosomal enzymes was observed. The activity was mainly located in and around lining cells and leucocytes in the pannus demonstrating increasing destructive characteristics. This resulted in marginal erosion and undermining of the cartilage border visible from Day 5 continuing gradually to total joint destruction after 5 weeks. The glycosaminoglycan depletion was observed at the surface of the cartilage at Day 2 and was total after 2 weeks.
Our infection model should permit comparison of different therapeutic measures.

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Experimental and clinical materials have demonstrated the importance of early treatment of septic arthritis (Orchard & Stamp 1968, Goldenberg & Cohen 1976, Ho & Su 1982); progressive joint destruction has been reported in infections continuing for more than a few days (Bardenheier et al. 1966, Orchard & Stamp 1968). However, the time of irreversible cartilage changes has not been defined. We have developed a rabbit model of septic arthritis to study the course of the untreated condition.

Materials and methods

Eighty-eight rabbits (New Zealand white type SsC:CPH) weighing 3 to 4 kg were used for the experiments. The rabbits lived in laboratory cages with an ad libitum supply of water and standard food pellets. Daily veterinary control and weighing every second day were carried out.

Both knees had 0.5 ml of sterile saline injected through the patellar ligament via a 26-gauge needle on one side with the addition of about 10^3 *Staphyloccoccus aureus*, phage type 3C, obtained by blood culture from a case of septic arthritis. The bacteria were stored in a glycerol-broth solution at −70°C. Before each day of experiment, the bacteria were subcultured on 5 per cent blood-agar plates at 37°C. For the inoculum portions, 5–10 colonies were suspended in 3 ml sterile saline to a density of 0.35–0.40 at 540 nm (Model 252 Corning Colorimeter). Appropriate dilutions of this solution provided a dose of approximately 10^3 colony-forming units/ml; serial tenfold dilutions were subcultured onto 5 per cent blood agar-plates for determination of viable counts.

No treatment was instituted. The animals were killed by an overdose of Nembutal in 12 groups of six animals up to 5 weeks after inoculation. For longer observations, 4 groups consisted of only 2–4 animals. One animal died of septicemia after 9 days and 2 never developed a joint infection, thus leaving 85 paired knees for evaluation. Just prior to killing the rabbits, the intraarticular temperature was measured in 60 rabbits with a needle electrode (thermocouple probe type TE 3-S, Ellab a/s, Denmark) placed between the femoral condyles in both knees and compared with the rectal temperature.

Immediately after death, cultures were taken from the joint fluid at surgical exposure, and the gross pathology estimated with particular reference to inflammation/abscess formation in the synovial membrane, ligament laxity, and erosion or destruction of the articular cartilage.

Both knee joints were removed and fixed in buffered formaldehyde, decalcified in formic acid and embedded in paraffin. Slices, 6 μm thick, were cut from the patella and stained with hematoxylin-eosin and safranin-0-iron hematoxylin-fast green, in orthochromatic form, for histologic study. A scoring system developed for septic arthritis (Salter et al. 1981) was used for the microscopic assessment of cartilage changes (Table 1).

Unfixed synovial tissue was immediately frozen with CO_2 ice (-70°C) for later estimation of proteolytic enzyme reaction. Acid phosphatase activity was used as indicator for semiquantitative demonstration of

Table 1. Histologic-histochemical scoring (Salter et al. 1981)

	Score
I. Cellularity of cartilage	
Normal	0
< 10%	1
10%–25%	2
> 25%	3
II. Loss of matrix (erosions)	
Normal	0
< 10%	1
10%–25%	2
> 25%	3
III. Cloning of chondrocytes	
Normal	0
< 10%	1
10%–25%	2
> 25%	3
IV. Adhesions (pannus)	
No adhesions	0
Covering only margins of cartilage	1
Covering <50%	2
Covering >50%	3
V. Orthochromasia with Safranin-O	
Normal	0
Slight and patchy loss	1
Moderate loss	2
Severe loss	3

the enzyme reaction. The unfixed sections were incubated with 0.5 mg/ml naphtol-AS-BI-phosphate (Sigma N22509) as substrate, and hexazotized pararosanilin (Sigma P37507) as indicator (Barka & Anderson 1963). The examinations were performed by light microscopy at a magnification of x50 in serial sections from both infected and uninfected synovial tissues. The incubation was carried out on the unfixed sections at a temperature of 25°C and at pH 5.0. The time from incubation to occurrence of the first color reaction, which was seen as a distinct punctate reaction corresponding to the lysosomes (Reimann & Christensen 1979), was recorded with reference to the control knee value and termed the "initial time." All specimens in this investigation were examined blindly by one of us (PR-N).

Results

Signs of septic arthritis with fever, a hot tender joint, and swelling was pronounced after 2 days. A loss of up to a quarter of the body weight during the first 2 weeks was encountered. After this time, weight was gained, apart from 8 rabbits, which developed marked abscess formation along the femur at an early stage. A flexion contracture of about 120 degrees, the resting position, was apparent in all septic knees after 3 weeks.

The mean intraarticular temperature was 37.2°C on Day 1 and increased to 40.4° on Day 14 and slowly decreased to 39.0°C on Day 94. The temperature varied with the slightly higher rectal temperature and paralleled the temperature in the control knee, which was never exceeded by more than 2 degrees.

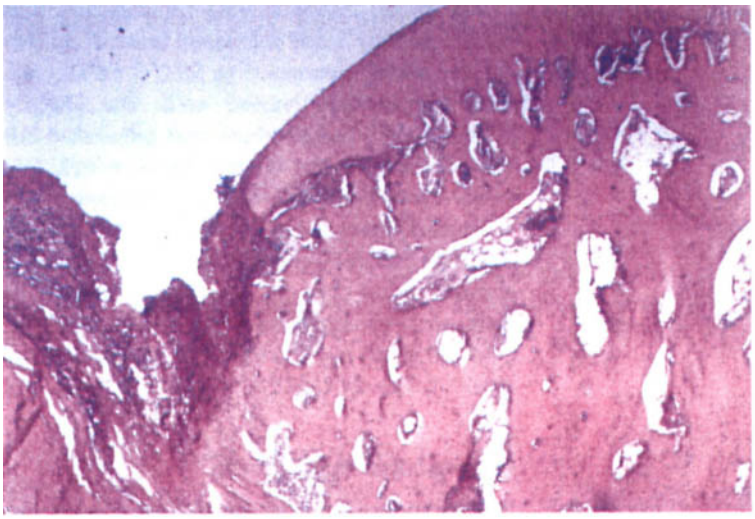
Bacteria were never grown from the blood or from the control knee. Exuberant growth of *S. aureus*, phage type 3C, was found in the exudate and synovial membrane taken from the infected joints in all rabbits but one with negative cultures on Day 94.

Macroscopic appearance.

The exudate was initially thin, but after the third day a creamy exudate was present with 1–9 cm increase in joint circumference. The pathologic changes were gradually worsened throughout the course (Figure 2). After 7 days, the cartilage softened with small fissures seen in three knees out of six and marginal erosion and pannus ingrowth at the femoral condyles from Day 11. The cartilage surface became very irregular, especially on the weight-bearing parts. On Day 17 the joint capsule had been eroded in 3 animals with destruction of the collateral ligaments, abscess formation in the thigh, osteitis with periarticular cortical destructions, and pus in the marrow canal. Increasing destruction of the cartilage mostly appeared at the weight-bearing areas of the joint. Fibrous anchylosis and total destruction of all the ligaments and cartilage was evident after 5 weeks.

Microscopic appearance

In the control knee, only a minimal inflammatory reaction was seen in the synovial membrane for 2 days after injection of physiologic saline. In the septic knees only lining cell proliferation and acute inflammatory reaction were initially evident (Table 2). From Day 5, the synovial inflammation was enormous with extension below the cartilage interface, loosening this in the marginal area (Figure 1). An intraosseous vascular congestion occurred diffusely through the underlying bone. The glycosaminoglycan-depletion was now clearly ob-



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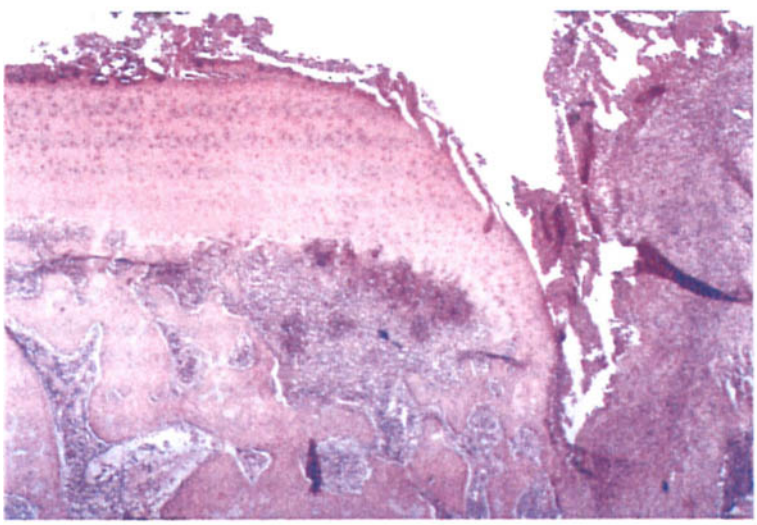

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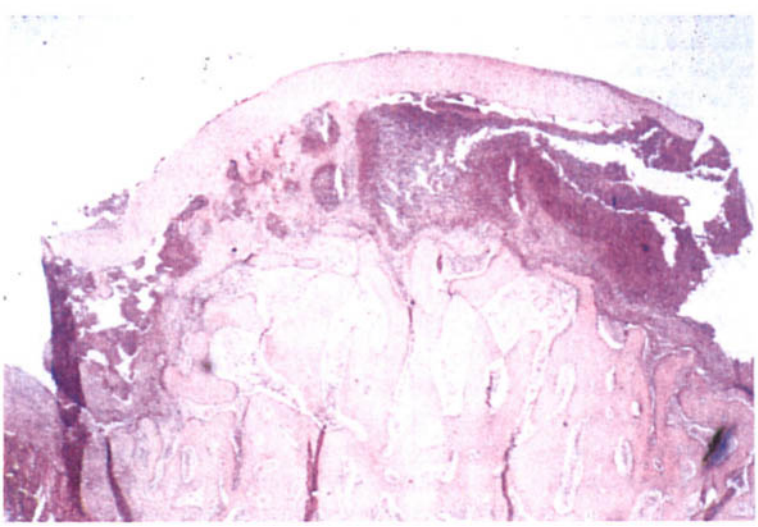

C

Figure 1. Septic knee arthritis in the rabbit.
A. Day 5. Marginal erosion and undermining of the cartilage border (HE, x7)
B. Day 17. Subchondral infection with marginal loosening and fissuring of the cartilage. Cloning is visible. (HE, x20).
C. Day 21. Only a few chondrocytes are visible and the matrix depletion is severe with "lifting off" of the cartilage. (HE, x10).

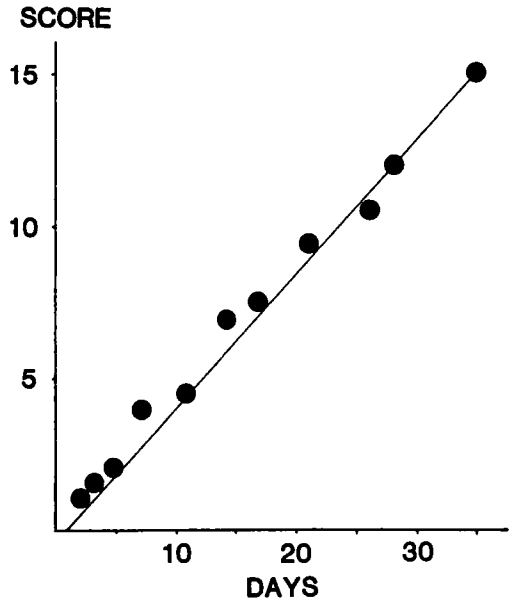

Figure 2. The progressive destruction in septic arthritis indicated by the scoring system of Salter et al. (1981).

served as a diffuse loss of staining, most prominent in the marginal areas. The synovial inflammatory front showed erosion of the marginal cartilage, and from Day 7, small fissures were evident on the cartilage surface in four of six knees.

From Day 11, the cartilage height had decreased by about 10 per cent, with depletion of chondrocytes. The loss of matrix was most distinct in the weight-bearing area of the cartilage. In all knees the cartilage surface was now irregular and fissured with a severe loss of safranin staining. After 2 weeks the cartilage surface was very irregular with further marginal erosion. The subchondral inflammation extended all the way along the bone-cartilage border with vascular crossing of the tidemark. No safranin staining could be identified, indicating total depletion of glycosaminoglycans. In a few sections, cloning of the chondrocytes was observed together with increasing loss and fissuring of the matrix.

From Day 17, the cartilage destruction advanced steadily, with further increased erosion by pannus ingrowth and fragmentation reaching total destruction in all the involved knees after 5 weeks (Figure 1). The joint capsule could not be identified after 3 weeks, and growing calcifications were found in the periarticular tissue. The progressive joint destruction was reflected by increasing scores (Figure 2).

During the course of infection, staphylococci could be identified in the synovial membrane and pannus tissue. Most of the bacteria were phagocytized and appeared within the inflammatory cells, but occasionally the organisms were lying in extracellular clusters, most numerous near the cartilage.

Enzyme reactions

On the first day, the mean initial reaction time was 0.84 with 1.0 in the control knee. From Day 2, the mean time was 0.35–0.54, indicating severe release of lysosomal enzymes. The activity was mainly located at and around the lining cells and leucocytes in the pannus.

Discussion

The rabbit knee joint is suitable for and susceptible to experimental staphylococcal infections (Nagel et al. 1966, Salter et al. 1981). Our infection model proved to be reproducible and reliable since only 1 animal died of septicemia and only two joints did not develop arthritis. The bacteria chosen had thus proved its virulence.

The intraarticular temperature was never elevated more than 2 degrees centigrade above the temperature in the control knee; the highest temperature measured was 40.4°C. Curtiss & Klein (1963) found that thermal denaturation of glycoaminoglycans and collagen, at the temperature level observed in our experiments, occurred at a slow rate without significance for clinical cartilage destruction. In contrast, Harris & McCroskery (1977) found that human cartilage collagen was degraded three times faster by collagenase at 39°C.

The acid phosphatase activity measured semiquantitatively and expressed as the "initial time" was raised after 24 hours and remained at high levels throughout the experiment. At an early stage, the activity appeared intensely in the surface layers of the synovium and in the pannus, corresponding to the electron microscopic studies by Bhawan et al.

(1973). A similar lysosomal activity is found in rheumatoid synovium, which has a number of enzymes capable of cartilage destruction, including acid phosphatase (Dingle 1973, Harris et al. 1969) localized in the synovial lining cells (Kar et al. 1976) and the cartilage-pannus interface (Kobayashi & Ziff 1975).

Like Schurman et al. (1977) we observed two destructive processes acting simultaneously in the infected joint. The first occurred in the contact area between the synovial membrane and the cartilage. The inflamed and hypertrophied synovial tissue developed into an invading pannus, eroding and undermining the cartilage. This was evident already on Day 5, and continued with progressing erosion and lifting off of the cartilage, which was virtually floating on pus after 3 weeks. Although a powerful phagocytotic activity is initiated by synovial cells and heterophils with a marked initial clearance of organisms (Bhawan et al. 1973, Nagel et al. 1966), staphylococci are able to destroy leucocytic cell walls (Johnson et al. 1970). The release of bacteria in the pannus front may thus maintain the inflammatory reaction and the following lysosomal discharge, favoring the progressing marginal erosion. The subchondral vascular proliferation broke the tidemark after 2 weeks, a sign that is observed also in advanced osteoarthrosis (Mankin et al. 1971).

Simultaneously, a more universal reaction occurs in the infected joint. This was observed as an absence of safranin-staining ability, which in orthochromatic form is specific for glycosaminoglycan depletion (Rosenberg 1971). As in the series of Daniel et al. (1973), this depletion was noted from the surface of the cartilage after 2 days, as compared with the uninfected knee, and was significant after 5 days. In our investigation the depletion was much more pronounced in the marginal areas of the cartilage near the pannus front corresponding to the high local enzyme activity. The depletion progressed gradually and was total after 14 days. Cartilage depleted of protein-polysaccharide is destroyed through an enzymatic effect caused by collagenase released from the lysosomes (Weissmann et al. 1969, Schurman et al. 1977). The purulent material and bacteria obviously has no effect by itself in cartilage dissolution (Curtiss & Klein 1963), but merely seems to act as a provoking mechanism. Furthermore, the glycosaminoglycan-depleted cartilage is mechanically weakened, facilitating fibrillation and fragmentation (Harris et al. 1972). In our investigation the cartilage surface was soft after 1 week and irregular on Day 11, from which period increasing loss of cartilage matrix was observed ending with fragmentation or erosion to subchondral bone after 5 weeks.

The histologic-histochemical scoring system (Salter et al. 1981) proved valuable for evaluation of the progressing changes in septic arthritis (Figure 4). The system is, however, based on a subjective technique, and thus has to be accepted with caution.

The first major cartilage changes were evident on Day 7 in contradistinction to 3 or 4 weeks in similar experimental arthritis caused by either *S. aureus* or *E. coli* (Bardenheier et al. 1966, Daniel et al. 1973, Schurman et al. 1975 a, b). Unfortunately, it is not known whether or not all of these test bacteria were virulent also to humans, thus explaining the different sensibility of the joint. In our series the microscopic appearance indicates that there is a point of irreversibility 5 to 7 days after introduction of the *S. aureus* to the joint, when the first signs of marginal erosion and undermining of the cartilage were visible. This corresponds to 2–4 points in the histologic-histochemical scoring system.

Experimental and clinical studies thus indicate that the treatment of septic arthritis has to be started so early that the joint becomes sterile before 5 days (Goldenberg & Cohen 1976, Newman 1976, Orchard & Stamp 1962) in order to stop the inflammatory reaction to cause permanent changes. Further, our infection model is reliable and seems useful for comparison of different therapeutic measures.

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