Subchondral bone formation in arthrosis

Polychrome labeling studies in mice

Joachim Benske, Michael Schünke and Bernhard Tillmann

We used polychrome sequential labeling to study the dynamics of subchondral bone sclerosis during developing arthrosis in knee joints of male STR/1N mice. This technique, using four different colored vital markers, gives detailed information about the site and time of new bone formation in the subchondral bone. In arthrotic joints, we found fluorescent bands arranged excentrically around the marrow cavities always pointing towards the cartilage lesions. The linear separation between the first label and the anatomic surface of the bone marrow cavity varied considerably between the experimental groups. In arthrotic joints, we found bone growth rates 3–4 times greater than in control joints. We also found that the degenerative process in cartilage and subchondral bone was a local phenomenon, because in areas with normal cartilage next to the sclerotic lesions appositional bone growth rates were unaffected.

Nearly all inbred strains of laboratory mice suffer from arthrosis, although the incidence, severity, and location of the articular lesions vary considerably between strains (Silberberg and Silberberg 1941, 1950, 1962). The pathogenesis of the murine form of the disease has been described by Sokoloff (1956), who found that the knee joint was most commonly affected. Regarding all the examined strains, the highest incidence is seen in male animals of the STR/1N strain (Sokoloff and Jay 1956).

According to experiments with the closely related STR/ORT strain (Walton 1977a, b, c, and 1979), arthrosis in aging mice much resembles corresponding lesions in man. Subchondral sclerosis in arthrotic knee joints of affected mice can be followed by using radiographic and histologic methods at different stages of the disease (Schünke et al. 1988).

We report the use of polychrome sequential labeling (Rahn 1976) for studying the dynamics of subchondral bone formation. This method permits (Gördes and Walcher 1976, Rahn et al. 1980) histologic analysis of growth and adaptation in subchondral bone during the development of arthrosis.

Institute of Anatomy, Christian-Albrechts University, Olshausenstrasse 40, D-2300 Kiel 1, Federal Republic of Germany

Correspondence: Dr. M. Schünke

Materials and methods

Totally, 56 mice aged 2–12 months were used: viz., 40 males of the inbred STR/IN strain, and as controls 6 STR/IN females and 10 C_3H males. The three groups were kept in individual boxes in an air-conditioned room at a constant temperature of 22–23 °C, and were fed a standard laboratory diet (Altromin/fortified) with access to tap water ad libitum.

Aqueous solutions of the following commercially available fluorochromes were administered: xylenol orange (90 mg/kg body weight), calcein (20 mg/kg body weight), alizarine complexone (50 mg/kg body weight), and tetracycline (15 mg/kg body weight). Each of the different fluorochromes was injected intraperitoneally twice at an interval of 7 days in the following sequence: xylenol orange, calcein, alizarine complexone, tetracycline (Reverin®), and calcein. Appositional growth rates in cancellous bone were determined by measuring the distances between the first xylenol-orange label and the anatomic surface of the bone marrow cavity.

Ether-anesthetized mice were killed by vascular perfusion via the left ventricle first with 0.9 per cent saline, followed by a solution of 36 percent neutral formaldehyde (one part) and 80 percent ethanol (three parts) as the fixative. After careful preparation of both knees, they were postfixed for another 24 hours, dehydrated, and embedded in methyl methacrylate without decalcification.

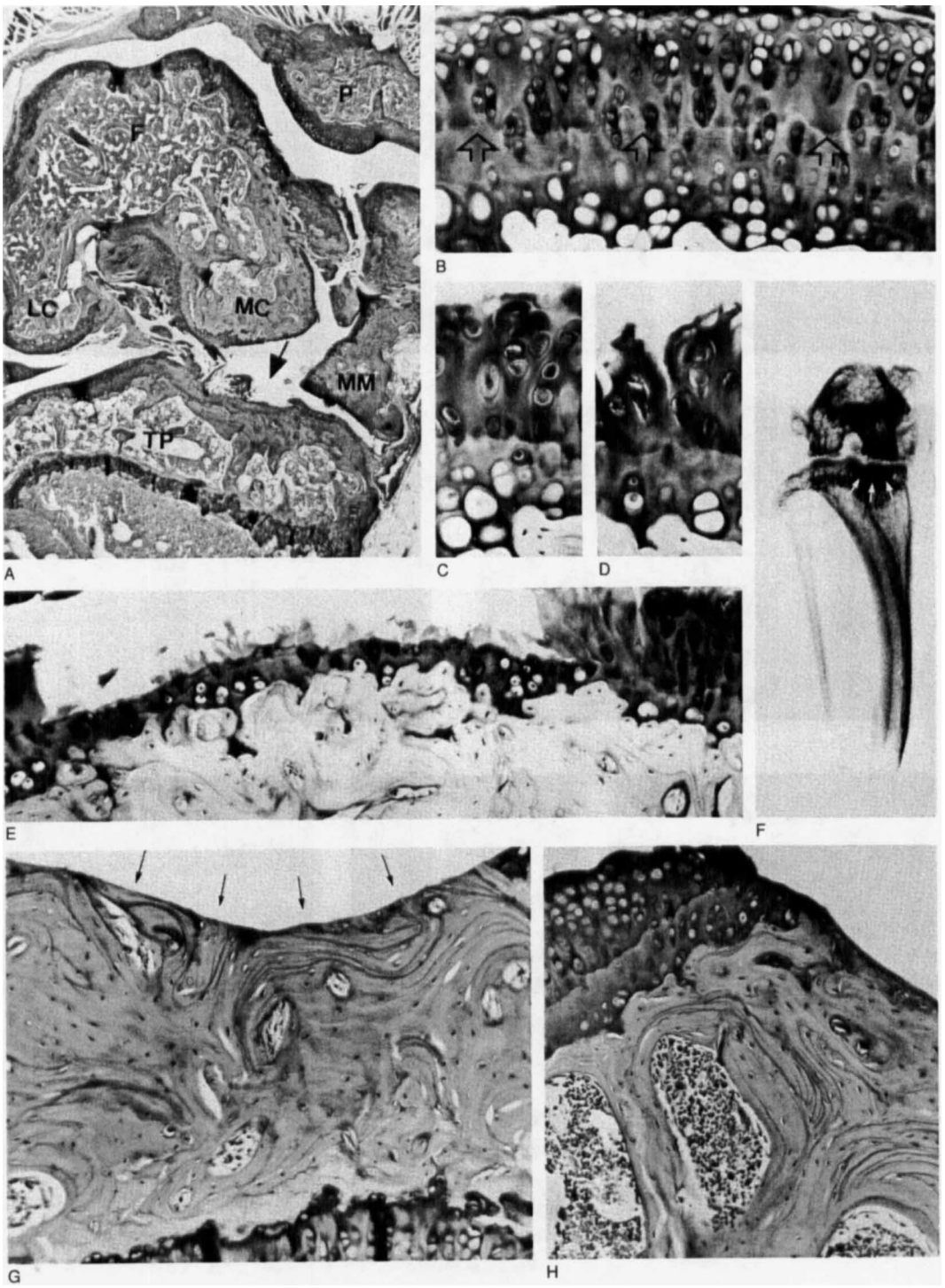

Figure 1. Morphology of developing arthrotic lesions in male STR/IN mice. Toluidine blue.

A. Frontal section of the right knee joint of a 5-month-old male STR/IN mouse. Black arrow indicates arthrotic lesions on the medial tibial condyle with incipient sclerosis (F) femur, (MC) medial condyle, (LC) lateral condyle, (P) displaced patella, (MM) medial meniscus, (TP) tibial plateau. x21.
B. Normal cartilage of the tibial plateau. (Arrows indicate tidemark). x225.
C. Fibrillation of cartilage. x225.
E. Significant loss of cartilage with partially exposed calcified layer. x180.
F. Radiograph of the right knee joint in an anterior-posterior view. (Arrows indicate subchondral bone sclerosis) x7.
G. Articular surface (black arrows) consists of bone. x150.
H. Transitional zone between severe arthrosis and healthy cartilage. Notice the marrow cavities below the intact cartilage. x150.

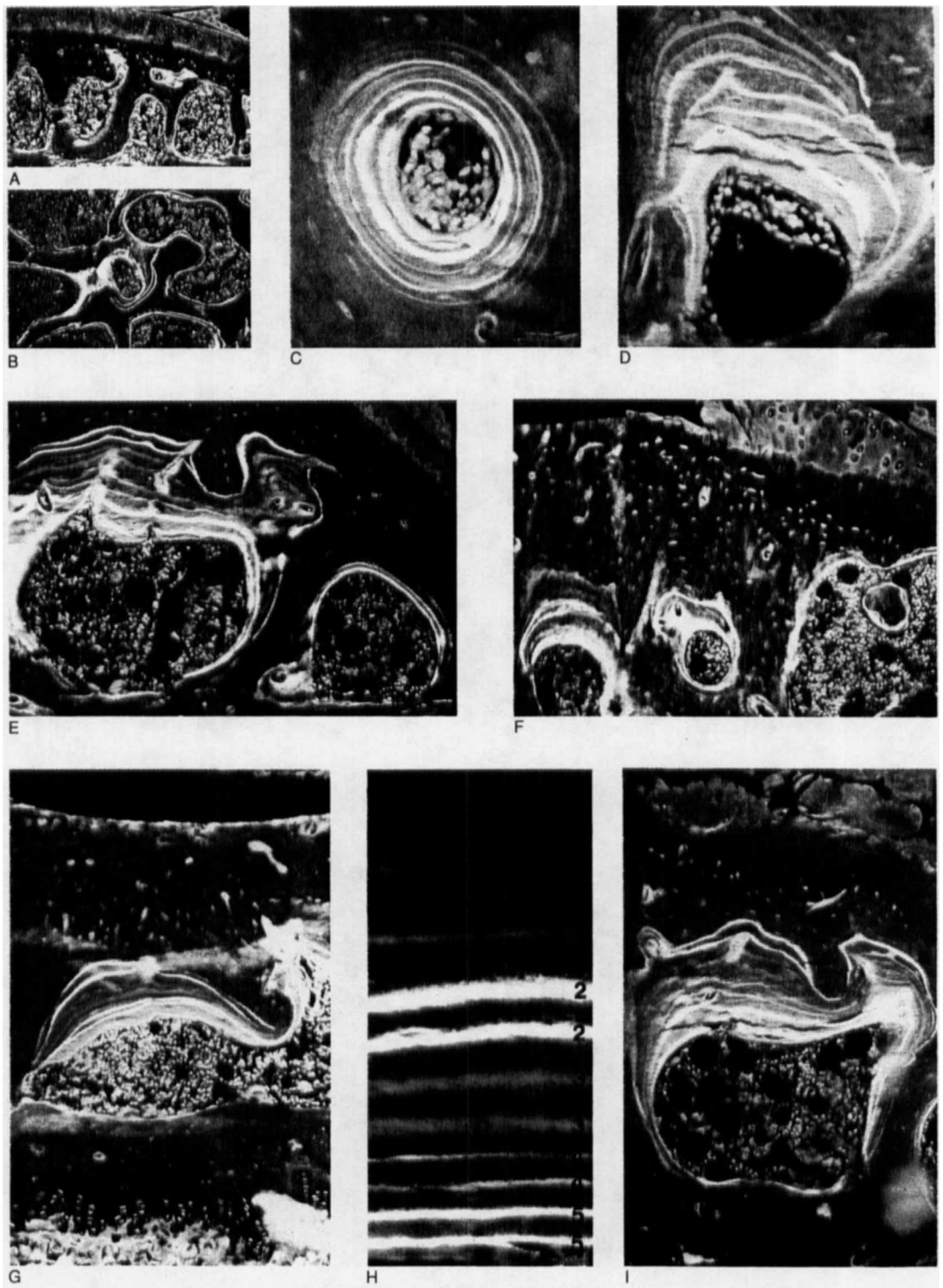

Figure 2. Polychrome sequential labeling of subchondral bone in normal and arthrotic STR/IN mice using four different colored fluorochromes (7-μm sections).

A. C_3H mouse, 3 months, male (bar = 190 μm), x55.
B. STR/IN-mouse, 3 months, female (bar =190 μm), x55.
C. C_3H mouse, 3 months, male (bar = 40 μm), x240.
D. STR/IN mouse, 8 months, male (bar = 40 μm), x240.
E. STR/IN mouse, 4 months, female (bar =100 μm), x100.
F. STR/IN mouse, 8 months, male (bar =100 μm), x100.
G. STR/IN mouse, 9 months, male (bar =100 μm), x100.
H. STR/IN mouse, 4 months, female (bar =20 μm); (1) xylenol orange, (2) calcein, (3) alizarine complexone, (4) tetracycline (Reverin®), and (5) calcein. x450.
I. STR/IN mouse, 4 months, male (bar =100 μm), x100.

Serial 7-μm sections were cut in the sagittal and anterior-posterior plane using a universal hard-cut microtome. After mounting on gelatin slides, the sections were pressed for 2 days at 37 °C, and every fifth section was stained with toluidine blue at pH 5.0. The slides were examined using a Zeiss microscope equipped with a fluorescence illuminator according to Ploem, using ultraviolet (BP 365) and blue light (BP 450–480) excitation. Micrographs were taken using Ektachrome 160 films. Radiographs were made using mammographic technique (37.5 RV, 80 mAs).

Results

Spontaneous degeneration of cartilage in male STR/IN mice appeared only on the medial tibial and femoral condyles, almost invariably leaving the lateral condyles unaffected (Figure 1). Continued loss of cartilage subsequently led to pronounced instability of the knee joint with a varus deformity, and was usually followed by medial patellar dislocation with arthrosis of the patellofemoral joint. Compared with the typical organization of normal cartilage, signs of early degeneration were slight superficial fibrillation followed by irregularly shaped fissures usually extending to the zone of calcified cartilage. The loss of uncalcified cartilage progressed to a stage where most of the articulating surface consisted of calcified cartilage at the level of the tidemark. At this time, subchondral sclerosis had already advanced to such an extent that it could easily be demonstrated radiographically.

The most severe arthrosis was recorded when at least two thirds of the articulating surface consisted of exposed bone, filling the whole epiphysis with sclerotic bone. Subchondral bone proliferation was always particularly pronounced immediately below the exposed surface, whereas in areas with normal articular cartilage next to the sclerosis, subchondral bone trabeculae were of normal appearance (Figure 1).

After administration of the different dyes over a period of 70 days, fluorochromes appeared as distinctly colored fluorescent lines in areas with active bone deposition (Figure 2). The distance between each of the bands corresponded to the amount of newly formed bone during the intervals between the injections. Generally, fluorescent material was found in epiphyseal trabeculae adjacent to the marrow cavities and on the endosteal surface of the cortical bone.

In control knees, we found regular bone deposition, especially along the various branching trabeculae surrounding the individual marrow cavities between the epiphyseal plate and articular surface. The newly formed bone was usually arranged concentrically around these cavities or around the various blood vessels in the subchondral bone.

Only in a few cases could individual fluorescence bands in the knee joints of control animals be distinguished from each other (Figure 2); most of the control animals displayed densely arranged bands, which did not allow accurate measurements between the bands. The rate of appositional bone growth was calculated to between 25 and 50 microns/70 days.

Knee joints with early arthrosis showed increased rates of appositional bone growth with a maximum up to 250 μ/70 days.

In advanced arthrosis the subchondral bone had a strong tendency to undergo focal sclerosis with thickening and confluence of trabeculae, forming massive blocks of bone containing single blood vessels. Compared with control animals, fluorescent bands in arthrotic knee joints were always arranged excentrically and were directed towards the cartilage lesion. In addition, arthrotic knee joints revealed bands that in most cases could easily be differentiated from each other.

In knee joints with advanced arthrosis, the maximal rate of bone growth was 100 to 150 μ/70 days (Figure 2). In areas with normal articular cartilage next to the sclerotic region, however, the growth rate was unaffected (Figure 2).

Discussion

Opinions differ concerning the cause or the consequence of subchondral bone sclerosis (Mohr 1984). Most investigators believe that articular cartilage is the primary site of the joint damage (Maroudas 1976, Sweet et al. 1977, Muir 1977, Greiling 1980, Tillmann 1980, Ali 1980b). Loss of hyaline cartilage and the following stress are considered to be the cause of the reactive sclerosis in the subchondral bone (Sokoloff 1980). It is still open to speculation whether the damage of articular cartilage is caused by altered degradation or altered synthesis of extracellular matrix. Here, collagen fibers (Gay et al. 1976, Ehrlich et al. 1977), as well as proteoglycans (Mankin et al. 1981, Muir 1977), can be involved. Others, however, believe that an increased hardness of subchondral bone (Radin et al. 1970, 1972), changed vascularization (Stephens et al. 1979), or altered blood flow (Arnoldi and Reimann 1979) produces corresponding cartilage lesions.

The STR/IN mouse is a suitable model for the study of morphologic changes in arthrosis because of the high incidence of arthrosis and the short and limited period of necessary observation; the degeneration

starts as early as at the age of 2 months, and at 1 year 75 percent of the animals have advanced arthrosis. The small size of the knee joints enables serial sectioning and precise identification of the fluorescence bands. Thus, it is possible to study the uptake of fluorochromes in the entire joint. Moreover, this technique reveals additional histologic details, because fixation and embedding permit conventional histologic staining.

Tetracycline is the only fluorochrome that clearly inhibits bone growth (Rahn 1976). The other markers with the applied quantities do not substantially disturb bone formation (Rahn and Perren 1970, 1971, 1972), and the dosages used were far below toxic levels. The fluorescence is not decreased by embedding or by UV-light, which made polychrome sequential labeling possible in sections only 7 microns thick, whereas former investigations used a thickness of 50–100 microns. We found an increased, regionally limited proliferation of the subchondral bone in arthrosis; and the proliferation of subchondral bone depended on the extent of cartilage degeneration.

Our values for appositional bone growth rates are in general agreement with studies by Lee et al. (1965), Simmons and Kunin (1970), and Frost (1960), who calculated bone growth rates of 1.5–2, 3–4, and 1 μ for dogs, rats, and human beings, respectively. An increased metabolic activity of subchondral bone accords with results of Danielsson et al. (1963) and Andersson et al. (1967), who demonstrated a marked uptake of radioactive strontium in human coxarthrosis, particularly in regions with arthrosis. Havdrup et al. (1976) noticed the absence of osteoclasts in the corresponding regions of arthrotic knee joints and a decreased resorption of bone in areas of sclerosis. In our study the physiologic balance of apposition and resorption in the sclerotic subchondral bone was disturbed in such a way that increased bone formation obviously was facilitated by decreased bone removal.

Our results also indicate that the subchondral sclerosis may be caused by the cartilage lesion, and that even slight changes of the hyaline cartilage may initiate proliferation of the subchondral bone. In addition, degenerative processes in the cartilage and subchondral bone are obviously local phenomena, because in areas with normal articular cartilage next to the sclerotic lesions, the bone growth rate was unaffected.

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