CHONDROCYTE MULTIPLICATION IN OSTEOARTHRITIC ARTICULAR CARTILAGE

ALASTAIR G. ROTHWELL and GEORGE BENTLEY, OXFORD, ENGLAND

From the Nuffield Orthopaedic Centre, Oxford*

Chondrocytes in mature articular cartilage rarely divide. From colchicine studies in rabbits, Mankin (1964) suggested that the mitotic turnover time is in years rather than days or months. This means that the likelihood of observing a cell in mitosis in histological sections of articular cartilage is almost zero. However, under experimental conditions chondrocytes respond to mechanical or chemical injury by proliferation and by increasing the synthesis of matrix glycosaminoglycans (Chrisman 1969) and collagen (Repo and Mitchell 1971). These responses have been observed after laceration (Mankin 1962b, Meachim 1963, Mankin and Boyle 1967), sustained pressure (Trias 1961, Crelin and Southwick 1964), and intra-articular injections of papain (Bentley 1972). The changes are presumed to be a secondary effect triggered by degradation or loss of the proteoglycans of the matrix.

Great interest is centred on these studies in view of their relevance to the repair potential of articular cartilage, and particularly the possibility of inducing healing in the early stages of damage as in chondromalacia patellae, incipient osteoarthritis, or following meniscectomy. Repair of cartilage would require proliferation of cells as well as production of new matrix. Though matrix production appears to be vigorous in osteoarthritis, information on cell proliferation has been scant (Collins and McElligott 1960). In the late stages Mankin and Lippiello (1970) demonstrated a quantitative increase in uptake of tritiated thymidine, but qualitative demonstration of cell division in autoradiographs had not been demonstrated when this study was begun. Accordingly an investigation was carried out in an attempt to discover if the chondrocytes would take up tritiated thymidine, which could be demonstrated in autoradiographs and would indicate their division.

EXPERIMENTAL METHOD

Specimens of articular cartilage with attached subchondral bone, 5 millimetres in diameter, were removed by trephine from the superior and inferior surfaces of fresh osteoarthritic femoral heads removed during replacement arthroplasty. Care was taken to avoid cartilage over osteo-phytes and fibrous tissue on the surface of eburnated areas (Fig. 1). Each specimen was incubated in a roller tube at 37 degrees Celsius for twenty-four hours in the following solution: 1 millilitre of Eagles Basal medium, with 1) glutamine, 2 millimolars; 2) penicillin, 100 units; 3) streptomycin, 100 units; and 4) tritiated thymidine, 50 microcuries.

The specimens were then washed in medium, fixed in 10 per cent neutral formalin and decalcified in ethylene-diamine tetra-acetic acid (EDTA). Serial sections were cut 5 microns thick, and autoradiographs were prepared by the dipping technique (Joftes 1959), using Kodak NTB₃ emulsion. The slides were exposed in light-proof boxes for twenty-four days at 4 degrees Celsius. They were then developed and fixed using Kodak D19 developer and

* Requests for reprints should be sent to Mr George Bentley, Department of Orthopaedic Surgery, Nuffield Orthopaedic Centre, Headington, Oxford.
Kodak fixer. After washing in running tap water at 18 degrees Celsius for thirty minutes, the autoradiographs were stained with haemalum and eosin. Routine haematoxylin and eosin sections were also prepared from the same specimens.

**RESULTS**

Twelve specimens from six femoral heads were examined. Rich uptake of thymidine-$^3$H by subchondral marrow cells confirmed the efficacy of the technique (Fig. 2). The haematoxylin
and eosin sections confirmed the presence of osteoarthritis, with erosion of articular cartilage down to Zones II or III in the areas sampled.

Tritiated thymidine is taken up in the S phase of the mitotic cycle, and its presence as label over the nuclei of cells in autoradiographs is accepted by most authorities as an indicator of DNA synthesis and impending cell division (Taylor, Woods and Hughes 1957; Cronkite, Bord, Fliedner and Rubini 1959; Hughes 1959; Owen 1970). Mankin (1962a) demonstrated that thymidine-3H rapidly traverses cartilage matrix and labels those cells about to divide.

There was scattered labelling of chondrocytes in sections from four of the six femoral heads. Labelled cells were seen in both Zones II and III (Figs. 3 to 6). No more than four labelled cells were seen in any one section. Approximately 50 per cent of cells were found in typical chondrocyte clusters, but other labelled cells were quite isolated.
FIG. 5
An autoradiograph showing three labelled chondrocytes close together in Zone III. (×800.) (The translucent area is a bubble in the emulsion.)

It was not possible to correlate the frequency of labelled cells with the degree of cartilage erosion because the latter was so variable. It was of considerable interest that labelled cells were seen more often in superior segment specimens than in inferior segment ones, although there were insufficient numbers to make a definite statistical evaluation. This finding suggests that the weight-bearing areas show more cell division that the non-weight-bearing areas, which may indicate a more vigorous proliferative response to damage. This has been suggested by the findings of Mankin, Dorfman, Lippiello and Zarins (1971).

DISCUSSION

The ability of articular cartilage to repair must be confirmed before therapeutic healing can be achieved in joints damaged by intra-articular fractures, surgical trauma such as that inflicted at meniscectomy, infections, or varying degrees of osteoarthritis or rheumatoid arthritis.

In osteoarthritis Chrisman has described a cycle of breakdown, illustrated in Figure 7, whereby under the influence of pressure, chondrocytes are damaged and release cathepsins which cause degradation of the cartilage matrix; this produces or aggravates fissure formation, which exposes more chondrocytes to excessive pressure during joint movement and causes release of more cathepsins from damaged cells; further matrix breakdown ensues in a cycle of changes. This mechanism is probably the final common pathway of cartilage breakdown from any cause. In response to these changes an increased production of matrix glycosaminoglycans occurs (Chrisman 1969). As for collagen, which was previously thought to be inert in response to matrix depletion, recent work also demonstrates a potential for high turnover (Repo and Mitchell 1971).

Articular cartilage has a high matrix to cell ratio, however, and significant repair would require proliferation of cells as well as new matrix production. The present experiment gives direct histological evidence that proliferation of chondrocytes occurs in osteoarthritic cartilage.
Fig. 6
Above, an autoradiograph of labelled cells in Zone III; (i) mid zone, (ii) deep zone. (× 80.)
Below, a high-power photograph of (ii) showing its association with a cluster. (× 800.)
This ability to proliferate might be potentiated by drugs or hormones. There is evidence that in experimental conditions chondrocyte proliferation is enhanced by growth hormone (Silberberg, Silberberg and Hasler 1964; Leach and Chrisman 1971) and by "sulphation factor" (McConaghey 1972). In acromegaly chondrocyte proliferation occurs as part of a general connective tissue response to growth hormone (Bluestone, Bywaters, Hartog, Holt and Hyde 1971). Quite recently Hulth, Lindberg and Telhag (1972) from Sweden have recorded evidence of cell proliferation in osteoarthritic cartilage. Increased knowledge in this field may lead to control of the mechanism of cell proliferation and matrix production, and hence to controlled induction of repair in mature articular cartilage.

SUMMARY
1. Twelve trephine specimens of articular cartilage and subchondral bone taken from six fresh osteoarthritic femoral heads were incubated in a medium containing tritiated thymidine, and autoradiographs were prepared from serial sections five microns thick.
2. Scattered labelling of chondrocytes in sections from four of the six femoral heads was demonstrated. No more than four labelled cells were seen in any one section. About half were found in typical chondrocyte clusters.
3. The implications of this evidence of chondrocyte multiplication with regard to the repair of damaged articular cartilage are discussed.

We wish to acknowledge the advice and encouragement of Professor R. B. Duthie, in whose department this investigation was performed. Mr. D. Haynes and Mrs. Diane Dean gave valuable technical assistance. The illustrations were prepared by the Photographic Department of the Nuffield Orthopaedic Centre.

REFERENCES


