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ABSTRACT:

CONTROL OF CARTILAGE CATABOLISM IN RHEUMATOID ARTHRITIS

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Whilst our early studies at the Strangeways (DINGLE J.T., Review, 1971) on embryonic cartilage were the first to demonstrate a release of catabolic enzymes into the extracellular environment during tissue degradation, we have long been convinced that these studies indicated only one product of cellular catabolic activity rather than pointing to specific enzyme action on the matrix. It is likely that catabolism of matrix macromolecules occurs in situations, both intra- and extra-cellular in which the conditions of enzyme concentration, pH and ionic concentration are optimal for individual enzyme action. These conditions may well depend on cellular activity and hence the relative importance of a particular proteinase may also vary greatly, and may be very different in a local tissue microenvironment from that which obtains in an enzyme assay performed under optimal but artificial conditions.

In an attempt to overcome some of the difficulties in determining the precise mechanism of local catabolic activity, I developed a cavity organ culture system enabling the introduction of characterized molecules into a microenvironment which at least approximates to that inside degrading tissue. A cavity holding 10 µl of fluid is made inside a cylinder of cartilage (usually bovine nasal cartilage). After being filled with radiolabelled substrate, either purified (^{35}S)proteoglycan or (^{14})collagen the cavity is closed by a screw and the cartilage is then cultured for 4-10 days. At various times the contents of the cavity can be recovered quantitatively and either placed on small Sepharose columns to determine the molecular weight of the proteoglycan or run on sodium dodecyl-sulphate electrophoresis to determine the state of the collagen. At the same time that the radioactive substrate from the cavity is assayed, the degradation of the cartilage proteoglycan of the organ culture itself is also measured as an expression of the catabolic activity within the tissue.

Proteoglycan aggregates placed in the cavity for 6-24 h on Day 6 showed considerable degradation in tissues that had been caused to resorb by vitamin A, or "Catabolin" (see below)

as compared with controls. This in itself is perhaps not surprising, as one might expect that enzymes would be released that would degrade both the cartilage matrix and also the material artificially introduced inside the tissue. However, when these experiments are considered in conjunction with the so-called "hamburger" experiments (DINGLE, 1976) some interesting conclusions can be drawn. In these latter experiments the proteoglycan was placed inside an acrylamide gel of known pore size and then the gel was totally enclosed in a sandwich of cartilage. Under these conditions the cartilage proteoglycan was readily degraded but the release of proteoglycan from the gel insert was not increased. We have shown cathepsins D and B. Nevertheless it would appear that the substrate inside the acrylamide gel was not available to such catabolic enzymes, unlike the free substrate in the cavity organ cultures. The conclusion we draw from these experiments taken together is that enzymes capable of matrix degradation are in some way hindered or inhibited from acting in a soluble and diffusible form (in the cavity system the substrate would be free to diffuse to an enzyme site).

It has often been suggested that the breakdown of articular cartilage, observed in arthritis, particularly in the region of the pannus, is due to release of enzymes from the inflammatory cells or the invading synovial fibroblasts, perhaps even from the adjacent blood vessels. To investigate this relationship in cartilage resorption, Dame Honor Fell and her associates studied the effect of synovial tissue on cartilage in organ culture (FELL and JUBB, 1977). When the synovium was placed in contact with either living or dead (frozen-thawed) cartilage it caused severe depletion of the matrix; this was greater in the living than in the dead cartilage. If, however, the cartilage was explanted at a distance from the synovium, only the living cartilage was affected. From these results it was concluded that the synovium affected the cartilage in two ways: by a direct action on the matrix and by an indirect action mediated through the living chondrocytes. DINGLE (1979) obtained similar effects with synovial tissue from experimental arthritic rabbits cultured in annuli of bovine cartilage. Since we found that the addition of purified α_2-macroglobulin, which traps most proteinases, did not affect the resorption of cartilage induced by synovium placed at a distance from it, it was unlikely that active proteinases secreted by the synovium were the causative factors. We found next that the breakdown of matrix occurred in living cartilage cultivated in used synovial medium. Consideration of these experiments led us to suspect that the synovium was producing some substance, other than an enzyme, which was responsible for the breakdown of cartilage matrix. A search for a chondrocyte catabolic factor which might be secreted by synovial tissue was therefore initiated. Before this problem could be tackled on a biochemical scale it was necessary to devise a suitable biological assay. DINGLE and HEMBRY developed a method whereby discs of bovine nasal cartilage could be grown in organ culture and 100 -

150 cultures could be set up at one time. This procedure, in combination with Barrett's blue-dye method for assaying proteoglycan, allowed the assay of large numbers of fractions and made analytical and preparative chromatography a practical possibility.

This research is currently being carried out in collaboration with Drs. SAKLATVALA, TYLER and HEMBRY in the Strangeways and with Dame HONOR FELL and Dr. JUBB in the Division of Immunology at Cambridge University. Very recently we have isolated an active fraction which can cause over 80 % resorption of cartilage matrix when added in µl amounts to the bioassay culture system (DINGLE et al., 1979). These results have been confirmed on pig articular cartilage by Fell and Jubb in their Laboratory. They were given fractions from a column effluent and assayed them blind; they were able to confirm that activity occurred in the peak from the column. This column run was made on an ammonium sulphate fraction from which the collagenase and neutral proteinases had been removed. This material, provisionally named "Catabolin" (DINGLE et al., 1979) is not an active enzyme nor is it a serum constituent and it did not affect dead cartilage. It was effective on living human as well as bovine and pig cartilage, but not on embryonic chick material. It is thought to be a peptide of about 20,000 molecular weight (it is susceptible to proteolytic degradation) and since it can now be prepared in reasonable yield its characterisation is being actively pursued. The pathogenic significance of this catabolic factor will depend on whether we can repeat these results with human rheumatoid synovial tissue. Preliminary experiments suggest that we can; if this proves to be the case we would suggest that this material, secreted by abnormal synovium, acting directly on chondrocytes, may be the initial trigger for the resorption of articular cartilage in arthritis. If this is so then we may have some direct indication of an important pathway of degradation.

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