Heberden oration 1978

Recent studies on the control of joint damage: the contribution of the Strangeways Research Laboratory

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Some 100 years after Heberden's observations on rheumatic patients Dr T. S. P. Strangeways, the Huddersfield lecturer in special pathology at Cambridge University, decided that knowledge of chronic arthritis was still so sparse as to need a new research impetus. With the aid of a small group of colleagues he raised the funds and supervised the building of the Cambridge Research Hospital, which after his death in 1926 became known as the Strangeways Research Laboratory. The hospital, which admitted patients up to about 1920, was in its early years funded largely from Strangeways's own slender means. He was a man of considerable vision and it was in the early 1920s that he decided that basic knowledge of the biology of the disease process needed to be advanced before much could be done directly to aid the rheumatic patient. To this end he turned the research at the hospital in the direction of cell biology, which, after Strangeways's death, was so ably developed by Dame Honor Fell and her associates. During the next 30 years research at the Strangeways became known internationally for studies utilising the culture of cells, tissues, and organs. To some degree the original interests of Strangeways, while not forgotten, were left in abeyance while the techniques in tissue catabolism, which were to prove so fruitful in the last decade, were developed.

It was some 35 years after T. S. P. Strangeways's death that I joined Dr, later Dame, Honor Fell from the Royal National Hospital for Rheumatic Diseases in Bath, and was reminded by her that the staff of this hospital had been among Strangeways's earliest collaborators in his research into rheumatic diseases.

The application of biological techniques developed in the Strangeways Laboratory to the study of the mechanisms of joint damage has been the framework within which during the last 2 decades Dame Honor Fell and I have re-established research into arthritis in this laboratory. It is my intention in this oration

to review some aspects of the current state of knowledge of the catabolism of articular cartilage and to comment on the part played by the Strangeways staff in this research. It will not be possible to discuss all the many projects that are now being pursued in our laboratory, since currently there are over 50 people involved in connective tissue research, working at the molecular, cellular, tissue, and clinical levels of investigation. I will therefore confine my comments to 5 principal questions which we believe are capable of being answered and which we think will yield useful knowledge about the mechanisms of joint damage in arthritis.

Question 1: What is the chemical nature of the attack on the matrix?

It is now understood that resistance of articular cartilage to the stresses imposed by its work load is due to the interaction of 2 macromolecules, namely, collagen, the fibrillar element, and proteoglycan, the packing element. The continued integrity of these molecules is essential for the maintenance of the structure of the cartilage matrix and hence for the function of the articular surface. Even partial enzymic degradation of one or other of these molecules results in a loss of resistance to tensile or compressive stress (Kempson *et al.*, 1976). Our work leads me to believe that the initial enzymic action is on the proteoglycan component of the cartilage; the collagen is perhaps protected by the presence of high concentrations of glycosaminoglycans. The temporal relationship of proteoglycan degradation to collagen catabolism is illustrated in Fig. 1. A similar relationship is observed in cartilage from several species under a variety of conditions. The type of experiment illustrated in Fig. 1 is often associated with substantial breakdown of collagen and hence complete loss of structure of the cartilage. It seems likely that an initial loss of proteoglycan can be restored by the synthetic activity of the chondrocytes, but if subsequently the collagen is

Fig. 1 The figure shows the time course of release of proteoglycan and collagen, measured as hydroxyproline, from bovine nasal cartilage in organ culture; assayed in the presence and absence of 3 µg/ml retinol. The results are expressed as a % of the total matrix present in the organ culture. The assays were carried out for each two day period when the medium was changed (CPG = cartilage proteoglycan).

significantly degraded the joint is probably past hope of repair (cf. regeneration in organ culture (Fell *et al.*, 1976). The architectural framework apparently imposed by the fibrillar element cannot be resynthesised in a manner compatible with the retention of the mechanical properties of the articular cartilage.

The nature of the attack on both proteoglycan and collagen is considered to be proteolytic. The principal sites of degradation of the proteoglycan are believed to be in the polypeptide chain, though the possibility exists of enzymic attack in the hyaluronic acid binding region. Most of the known tissue proteinases, both acid and neutral, are capable of degrading this polypeptide chain. It was demonstrated by Morrison *et al.* (1973) and Roughley and Barrett (1977) that even 1 or 2 clips in this chain reduced the molecular size of the proteoglycan molecule sufficiently to allow it to diffuse out of the matrix.

For some years collagen degradation at neutral pH has been regarded as the province of the classical collagenase. This type of enzyme, first isolated by Gross and Lapiere (1962), attacks the triple helix specifically, giving the well-characterised three-quarter/one-quarter split. Recent work by Barrett's group (Starkey *et al.*, 1977; Barrett, 1978) at the Strangeways has demonstrated that at least one other enzyme, the neutrophil elastase, is also capable of attacking collagen at neutral pH, though in this case the site of action is almost certainly in the cross-link region. Once this degradation has occurred, the collagen denatures and becomes susceptible to the various intra- and extracellular tissue proteinases present both in cartilage and in

synovial tissue. Since the attack on the 2 major components of the matrix is thought to be proteolytic, the next question must concern the identity of the catabolic enzymes.

Question 2: Which tissue proteinases cause articular damage?

Fig. 2 summarises our present state of knowledge on the acid and neutral enzymes present in the 3 major tissues of the joint. The synovial membrane including the pannus, has a full complement of both acid and neutral enzymes. Thus, cathepsins B, D, and N are all capable of degrading proteoglycan with a pH optimum of between 5 and 6.5 and may also under optimum conditions degrade collagen (i.e., in an intracellular vacuole). The neutral metalloproteinases, including collagenase, are synthesised and secreted by synovial tissue in culture, though the enzymes cannot be isolated in appreciable activity directly from the tissue (Werb and Dingle, 1976). Serine proteinases also are present in low activity. Articular cartilage contains substantial activities of cathepsins B and D and also has in addition cathepsin F (Dingle *et al.*, 1977), which seems to have some specificity for proteoglycan degradation at pH 5. There are only trace activities of the neutral proteinases in articular cartilage. Synovial fluid, on the other hand, has considerable neutral proteinase activity. Of articular interest is the neutrophil elastase, which, as Barrett has shown, is capable of degrading not only elastin but also proteoglycan and collagen. This enzyme also shows some specificity for collagen type. Thus, type 1 collagen of skin, bone, and tendon is rather resistant to elastase action, though it is very sensitive to degradation by the classical collagenase. However, type 2 collagen from

MAJOR CATABOLIC ENZYMES IN JOINT TISSUE

TISSUE SOURCE	ENZYME	
	ACID	NEUTRAL
Synovium (pannus)	Cathepsins B,D,N	Collagenase Metalloproteinases Serine Proteinases
Articular Cartilage	Cathepsins B,D,F	Trace activities
Synovial Fluid	—	Elastase Cathepsin G Plasmin

Fig. 2 Acid and neutral enzymes in 3 major tissues of the joint

cartilage, which is relatively insensitive to collagenase is readily degraded by elastase. This could have important implications, particularly in the acute phase of inflammation, when polymorphs may be present in the synovial fluid in large numbers.

Recent work by Reynolds's group (Sellers *et al.*, 1978) at the Strangeways on neutral metallo-proteinases shows that the activity of the latent form of these enzymes may be separated into fractions which when activated show specificity for gelatin, collagen, and proteoglycan. The molecular weights of these fractions are substantially different and allow latent enzymes to be separated by ultra-filtration. Such activities are shown in Fig. 3. Further work by Reynolds *et al.*, has demonstrated that all these latent enzymes may be activated by 4-aminophenyl mercuric acetate (Fig. 4) but do not appear to be stored in the tissue in significant quantities; rather the enzymes are synthesised and released directly to the extracellular environment. The control of the local activity of these enzymes is obviously important in the dynamic equilibrium of the cartilage matrix, and the effect of both neutral and pharmacological inhibitory factors upon this equilibrium is discussed below.

Question 3: Where do these enzymes act and which cells and tissues are involved?
In the arthritic joint there are 3 main sources of enzymes capable of degrading the components of cartilage matrix. These are the synovial membrane

Fig. 4 Activation of latent rabbit neutral metalloproteinases by the inclusion of APMA at various levels in the assays for the 3 activities indicated.

Fig. 3 Chromatography of latent rabbit metalloproteinases on Ultrogel AcA44. Column fractions were assayed for the activities shown in the presence of APMA (1mM). No activity with any of the substrates was observed when APMA was omitted from the assays.

and its associated pannus, the articular cartilage, and the synovial fluid. In recent years interest in many laboratories has concentrated on the enzymes from the synovial membrane particularly collagenase. Less interest has been expressed in the action of the enzymes of the synovial fluid, principally because of the presence of high concentrations of enzyme inhibitors in the tissue. The role of the chondrocyte in degradation of cartilage matrix has, in my view, been somewhat neglected. Fig. 1 shows that isolated articular cartilage can resorb its own matrix, and in the next section of this oration I shall develop the theme that the chondrocytes of such tissues are capable not only of synthesis but, under pathological stimulation, may in fact be the principal agent of the degradation of their surrounding matrix.

While our early studies at the Strangeways (Dingle, 1962; Fell, 1969) 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 indicate one product of cellular catabolic activity rather than identification of a specific enzyme action on the matrix. I consider that it is likely that catabolism of matrix macromolecules occurs in situations, both intra- and extracellular, in which the conditions of enzyme concentration, pH and ionic concentration are optimal for individual enzyme action. These conditions may well vary depending on cellular activity, and hence the relative importance of a particular proteinase may also vary greatly from time to time. It follows that

the activity of catabolic enzymes, be they acidic or neutral, may be very different in a local tissue microenvironment from that which obtains in an enzyme assay performed under artificial conditions on external tissue fluid.

In an attempt to overcome some of the difficulties in determining the precise mechanism of local catabolic activity I have developed a cavity organ culture system which is shown diagrammatically in Fig. 5. The objective of the system is to introduce characterised molecules into a microenvironment which will at least approximate that inside degrading tissue. A small cavity holding 10 μ l of fluid is made inside a cylinder of cartilage (usually bovine nasal cartilage), and after being filled with purified, characterised, and radiolabelled substrate, either [35 S] proteoglycan or collagen labelled with 14 C, the cavity is closed by a screw. The small cylinder of cartilage is then cultured for 4–10 days. At various times the contents of the cavity can be recovered quantitatively by centrifuging in the shoulder tube shown in Fig. 6. The material from the cavity is placed on small Sepharose 2B or 4B columns to determine the molecular weight of the proteoglycan or run on sodium dodecylsulphate 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. Such measurements are shown in Figs 7 and

8. As may be seen in these figures, proteoglycan aggregate placed in the cavity between day 6 and day 9 showed considerable degradation in tissues that had been caused to resorb by vitamin A, as compared with controls without vitamin A. This in itself is perhaps not surprising as one might expect

RELEASE OF CPG FROM CARTILAGE MATRIX IN CULTURE (Co9)

Fig. 7 The figure shows the release of proteoglycan from bovine nasal cartilage grown in cavity organ cultures for 9 days in the presence and absence of 3 μ g/ml retinol. The results are the mean of 5 replicates. [35 S] CPG was introduced into the cavity between day 6 and day 9 (see Fig. 8).

SUB-UNIT CPG FROM CAVITY CULTURE. (Co9)

Fig. 8 Chromatography on Sepharose 4B of [35 S] proteoglycan from within the cavity organ cultures. The frozen/thawed tissue was incubated for the same period of time and under the same conditions as the tissue stimulated by retinol (3 μ g/ml).

Fig. 9 This figure shows the release of proteoglycan from the cartilage of controls and retinol-stimulated cartilage organ cultures, and also the release of [³⁵S]SO₄ from acrylamide gel inserts from within the same cartilage.]

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 I placed the proteoglycan inside an acrylamide gel of known pore size and then inserted the gel into a sandwich of cartilage in such a manner that it was totally enclosed. As can be seen in Fig. 9, the proteoglycan of the cartilage was readily degraded in these experiments, but the release of proteoglycan from inside the gel

insert was not increased. We have shown previously that the pore size of the gel was adequate to allow penetrations of enzymes such as 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 conclusions that I 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). Weight is lent to this hypothesis

Fig. 10 Localisation of cathepsin D in rabbit ear cartilage. The secreted enzyme was trapped by immunoprecipitation in the living cartilage; a second fluorescent antibody was used to localise the precipitation of sections of frozen tissue. The cells were counterstained with erichrome black.

Fig. 11 Localisation of cathepsin D in retinol-stimulated cartilage. Trapping and staining conditions were the same as for Fig. 10, but the cells were not counterstained. The cartilage was stimulated to secrete enzyme by culturing in the presence of 3 µg retinol.

by immunocytochemical work carried out in the Strangeways Laboratory (Poole *et al.*, 1976). In these studies specific antisera to several enzymes were raised, characterised, and used to trap secreted antigen in living cartilage. For example, Fig. 10 shows the pericellular release of cathepsin D by a single chondrocyte in unstimulated cartilage. It can be seen that the enzyme is localised as discrete "packets" at or very close to the cell surface. If the tissue is stimulated to resorb, this phenomenon is seen in the majority of cells (Fig. 11). In this experiment it is evident again that high concentrations of enzymes can persist at least for short periods of time in the pericellular environment of many cells. Experiments on human rheumatoid material made in collaboration with the staff of the Royal National Hospital for Rheumatic Diseases at Bath gave

similar results (see Figs 12 and 13). It may be speculated that the local concentration of enzyme, perhaps only for a short time, may be very high. If this is so and if the local pH and ion concentration are favourable, the catabolism of matrix molecules close to the cell surface may be very rapid. While these experiments do not prove that cathepsin D is the operative enzyme, recent work suggests that it is likely that similar pericellular concentrations of other proteinases may occur.

The above experiments lead me to suggest that the initial site of degradation of matrix is at or close to the membrane of the chondrocyte. This area, which I term the functional pericellular microenvironment, might be regarded as only a transitory site if the enzymes are diffusible, although the possibility of either acid or neutral enzymes actually associated

Fig. 12 Cathepsin D release from human rheumatoid tissue. *This figure shows the extracellular trapping of cathepsin D in an area of pannus erosion of human rheumatoid cartilage. The area taken for this study is shown in an adjacent section in Fig. 13.*

Fig. 13 Pannus erosion of human rheumatoid cartilage. (see Fig. 12)

with the cell surface cannot be overlooked. The physical presence of such microenvironments is not incompatible with the chondrocyte as viewed by high voltage electron microscopy (Fig. 14). The numerous fine processes and partial invaginations could allow enzyme activity to occur under optimum conditions which would not appertain at sites distant from the surface of the cell. In my view, therefore, stimulation or inhibition of the chondrocytes' catabolic activity might allow these cells to play a significant role in the degradation of cartilage matrix. The study of the factors that may control such chondrocytic function is likely to become very important in arthritis research.

Question 4: What are the mechanisms that control cartilage breakdown?

I will consider such mechanisms under two main headings: (1) control of enzyme action and (2) control of cellular functions.

INHIBITION OF CARTILAGE CATABOLISM BY INHIBITORS OF TISSUE PROTEINASES

These inhibitors fall into 2 classes, firstly, natural inhibitors present in the organism, and, secondly, synthetic inhibitors which have been used principally for studying enzyme mechanisms, but may possibly present means of pharmacological intervention.

Some inhibitors of the major tissue proteinases of joint tissue are shown in Fig. 15. The natural inhibitors with the exception of the tissue inhibitor of metalloproteinases (TIMP) are all serum or synovial fluid components. Among these, and perhaps one of the most interesting examples, is α_2 macroglobulin. This material, which has been studied in detail by Barrett and Starkey (1973), has a very wide specificity and is capable of combining with and inhibiting most of the known tissue proteinases. β_1 AC, which has been isolated by Woolley *et al.* (1976), is an inhibitor of collagenase, which may

modify enzyme activity in extratissue fluids. I doubt whether either these inhibitors or α_1 -plasmin inhibitor plays a significant role in preventing the digestion

Fig. 14 High voltage electron micrograph of a 0.5 μ m thick section of a rabbit chondrocyte. The tissue has been treated with a proteinase to create a microenvironment similar to that which may occur in actively catabolising cartilage. Magnification 13 300. Figure reproduced with permission of Audrey M. Glauert and H. Clarke Anderson (Glauert, 1974).

INHIBITION OF TISSUE PROTEINASES

ENZYME		NATURAL INHIBITORS	SYNTHETIC INHIBITORS
Neutral	Collagenase	α_2 M, β_1 AC, TIMP	EDTA Penicillamine
	Metalloproteinases		
	Cathepsin G	α_1 PI, α_2 M	(EACA) DFP
	Elastase		
	Plasmin		
Acid	Cathepsin B	α_2 M	Iodoacetate, leupeptin Pepstatin ?
	Cathepsin D	?	
	Cathepsin F	α_2 M	

Fig. 15

of extracellular matrix in the pericellular region, though they may play a more important part in 'looser' connective tissues such as the dermis. Nevertheless in cartilage it seems unlikely that inhibitors of relatively high molecular weight, such as α_2 macroglobulin, can reach sites of enzyme activity in sufficient concentration to prevent local activity. Thus, these inhibitors may be viewed as scavengers of released extratissue enzymes. However, these criteria probably do not apply to TIMP, which has been shown by Reynolds's group (Reynolds *et al.*, 1977) to be secreted by either the same cells as those that produce the metalloproteinases or at least by adjacent cells.

The concurrent secretion of inhibitor and enzyme results in an enzyme-inhibitor complex being produced which may be dissociated under certain conditions giving rise to active enzyme. The activated enzyme may be recombined with inhibitor isolated from a variety of tissue sources. Interestingly, Sellers and Reynolds (1977) have demonstrated that the time course of production of collagenase and collagenase inhibitor by a tissue may change during the course of an experiment. Thus, in organ cultures of rabbit calvariae the production of collagenase inhibitor falls during the experiment and then there is a rise in active enzyme (Fig. 16). On the basis of

Fig. 16 Production of collagenase and collagenase inhibitor by explants of fetal rabbit bone in culture. The culture medium was changed every second day and samples of the harvested medium were assayed for latent collagenase (O), active collagenase (Δ) and collagenase inhibitor ($\bullet$). Results are the mean $\pm$ SEM of 4 cultures (from Sellers and Reynolds, 1977).

these results the authors propose that the ratio of inhibitor to active enzyme is important in controlling both the physiological and pathological rates of tissue resorption.

Fig. 15 also shows some of the principal synthetic inhibitors of the tissue proteinases. Some years ago we found that it was possible to inhibit cartilage autolysis by the use of pepstatin. Nevertheless it is probably correct to say that, in spite of intensive investigation into the effects of these inhibitors on tissue catabolism, no inhibition of tissue degradation has been demonstrated in living tissue with any single inhibitor nor indeed with any combination of inhibitors. This might be taken to indicate that none of the enzymes listed in the table are in fact participating in the catabolic events. However, it seems more likely that the kinetics of inhibition are unfavourable. Thus, if the hypothesis of pericellular activity is correct, it is likely that the high local concentration of enzyme that occurs during secretion, coupled with the high concentration of substrate available, makes the conditions for inhibition not only difficult to understand (conventional enzyme kinetics applicable to dilute solutions would certainly not apply in these circumstances) but also would be very difficult to achieve. We have been able to demonstrate that labelled inhibitors such as pepstatin do penetrate cartilage and probably reach a concentration similar to that in the extracellular fluid, but this does not preclude the possibility that the concentration is insufficient to inhibit the local activity of high concentrations of active tissue proteinases. It is hoped that these problems can be resolved by the use of the cavity organ culture methods.

ACTIVATION OF ARTICULAR CARTILAGE CATABOLISM

It has been shown over a number of years that embryonic and adult cartilage can be stimulated to resorb by such exogenous factors as vitamin A (Fell and Mellanby, 1952), complement-sufficient antisera (Fell, 1975), and hyperoxia (Sledge and Dingle, 1965). These factors, while shedding light on pathways of chondrocyte activation, do not necessarily indicate the means by which articular cartilage is stimulated to resorb in rheumatic diseases, as for example in pannus erosion or in the extra-pannus thinning of cartilage. It has been suggested that the breakdown of cartilage, 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 associate studied the

effect of synovial tissue on cartilage in organ culture (Fell and Jubb, 1977). When the synovial membrane 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 membrane, only the living cartilage was affected. From these results it was concluded that synovial membrane affected the cartilage in 2 ways: by a direct action on the matrix and by an indirect action mediated through the living chondrocytes. Partial breakdown of the matrix occurred in living cartilage cultivated in used synovial medium (Jubb and Fell, in press). I obtained similar effects (Dingle, 1976) with synovial tissue from experimental arthritic rabbits cultured in annuli of bovine cartilage (Fig. 17) (Dingle, 1976). 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 proteases secreted by the synovial membrane were the causative factors.

Consideration of these experiments led me to suspect that the synovial membrane was producing some substance, other than an enzyme, which was responsible for the breakdown of cartilage matrix in used synovial medium. I therefore initiated a search for a chondrocyte catabolic factor which might be secreted by synovial tissue. Before this problem could be tackled on a biochemical scale it was necessary to devise a suitable biological assay. I 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 (Fig. 18). This procedure, in combination with the recent blue-dye method for assaying proteoglycan (Barrett *et al.*, in preparation), 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

ASSAY OF SYNOVIAL FACTOR USING BOVINE NASAL CARTILAGE

Fig. 18 The effect of different amounts of cartilage catabolic factor (SF) on the release of proteoglycan from cartilage discs in organ culture.

Fig. 17 The release of cartilage proteoglycans in the presence of rabbit synovium. Annuli of bovine nasal proteoglycan were grown in organ culture. The centre of the annulus contained synovial tissue from experimentally arthritic rabbit knee joints, normal synovium, or were left empty. The release of proteoglycan is expressed as a % of the total and was measured after 8 days in culture.

Fig. 19 The separation of cartilage catabolic factor on DEAE is shown. The peak containing one major and some minor protein bands was isolated and found to contain the active factor.

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 to μ l amounts to the bioassay culture system. These results have been confirmed on pig articular cartilage by Fell and Jubb in their laboratory. They were given fractions from the column effluent shown in Fig. 19 and assayed them blind; they were able to identify the peak shown in this figure as containing the active material. This column run was made on an ammonium sulphate fraction from which the collagenase and neutral proteinases had been removed. This material is not an active enzyme nor is it a serum constituent and it did not affect dead cartilage. It was effective on human as well as bovine and pig cartilage but not on embryonic chick material. It may be a peptide of some type (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. One preliminary experiment suggests that we can. If this proves to be the case, I would suggest that this material, secreted by abnormal synovium, acting directly on chondrocytes, may be the initial trigger for the resorption of joint cartilage in arthritis. If this is so, then we may at last have some direct indication of an important pathway which might be susceptible to pharmacological intervention.

Question 5: What pharmacological control is possible?

The inhibition of inflammation has been a major research topic in many laboratories for over 2 decades. In spite of intensive and wide ranging

research few really effective compounds have been discovered. The steroids, which were the first clinically important agents to be found, are still some of the most useful in controlling inflammatory reactions. However, one of the problems of these

The effect of liposome incorporated cortisol upon an acute experimental arthritis of 4 days duration

Fig. 20 The effect of liposomes containing cortisol palmitate on the knee joint of experimentally arthritic rabbits was measured by thermography. The results are the mean of 5 animals.

drugs has always been that of side effects. This is due at least in part to the necessity of using relatively high concentrations. Even when steroids are used locally the physician often finds it necessary to introduce as much as 50 mg of drug into a knee joint. In such a situation if the amount of drug is calculated on a tissue basis it is evident that the concentration to which individual cells are exposed is considerable. It seemed to me likely that one way to overcome side effects and to reduce undesirable cellular concentration was to attempt to target drugs directly to the cells it was desired to treat. The joint cavity seemed an ideal situation in which to attempt this. I do not intend to review here our studies on liposome-encapsulated steroids, as this has already been done elsewhere (Dingle, 1978),

but I will comment briefly on the preliminary clinical studies that have been made with this type of drug delivery. These pilot studies arose from years of collaboration between the Sybil Eastwood Arthritis Department at the Strangeways, the Rheumatology Department at Addenbrooke's Hospital, and Imperial Chemical Industries.

The object of this work was to prepare a stable microparticle containing the appropriate amount of steroid, which when phagocytosed by or fused with the synovial lining cells would cause just sufficient metabolic changes to alleviate the arthritis. Our collaboration resulted in a steroid formulation (hydrocortisone palmitate) in dipalmitoyl liposome which was effective in the treatment of experimental arthritic animals (Dingle *et al.*, 1978) (see Fig. 20)

Fig. 21 The figure shows the effect of the dose of liposome-encapsulated cortisol on experimental arthritis. The effect is expressed as difference in temperature from control knees. The results are the mean of 5 animals.

Fig. 22 The results show the effect of injection of liposome-encapsulated cortisol palmitate (2 mg) into the knee joints of acutely arthritic patients. The results are the mean of 6 patients. Four parameters of joint function and inflammation are shown. Patients were observed for 2 weeks.

The steroid encapsulated in these liposomes was active in the experimental animals when administered in as little as 2 µg per joint (Fig. 21). These experiments and others in which microcrystals of steroid were used convinced us that this method of treatment was worth investigating in the human rheumatoid patient.

Dr Hazleman and his associates at Addenbrooke's Hospital undertook a small pilot study, and the results obtained on 6 patients are recorded in Fig. 22. As can be seen, the single intra-articular injection of what, in our terms, was thought to be a low level of steroid, i.e., 2 mg per knee joint, substantially diminished the inflammation as judged by a number of criteria, both objective and subjective. These results have encouraged us to continue our basic studies on drug encapsulation and in particular to concentrate on preparations which might give longer activity in the patient.

Conclusions

I have posed some 5 questions and indicated how research at the Strangeways Laboratory has progressed in attempting to find answers to them. In work both here and elsewhere it has been shown that proteolytic cleavage, initially of proteoglycan and secondly of collagen, is responsible for the damage in the articular cartilage of the arthritic patient. I consider that the enzymes involved are almost certainly a combination of metalloproteinases and cathepsins. The extent to which each enzyme is quantitatively important is not yet known, but it may well vary both spatially and temporarily depending upon local conditions. I think it likely that these enzymes work principally in the pericellular environment of the cell and that the chondrocyte may be the key cell in causing breakdown of cartilage matrix. I would suggest that this is a physiological mechanism, perhaps important in the normal remodelling of cartilage, and under pathological stimulation the shift in the dynamic equilibrium between synthesis and degradation of matrix by chondrocytes is caused by local increase in active enzyme secretion. The control of such chondrocyte function may be due at least in part to the release from adjacent synovial tissues of factors, provisionally named 'catabolins', that can act directly on cartilage cells. How the production of synovial catabolin is controlled is totally unknown, but research into this question might well prove to be of crucial importance to the future regulation of joint disease. It may be that pharmacologically active compounds modify the secretion of such material by inflammatory tissues. If this proves to be true, development of the targeting of drugs to

specific cells, by such means as liposomal encapsulation, may prove useful in the treatment of both the acute phase of inflammation and the damage to the articular cartilage.

I hope I have shown that research into the catabolism of articular cartilage has moved from the description of the biochemical changes, via studies on the isolation, properties, and localisation of the degradative enzymes to the investigation of the control of cellular functions that mediate the pathological damage. Work in Dr Strangeways's Research Hospital and elsewhere during the coming years should prove to be exciting and important, and could yield information that the physician may be able to utilise in controlling these painful and crippling diseases.

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