Mechanisms of Connective Tissue Degradation

Periodontal disease, which is characterized by progressive destruction of the supporting structures of the teeth, is the major cause of tooth loss in the adult population. Since the supporting structures of the teeth are primarily composed of connective tissues, the National Institute of Dental Research invited 19 investigators to participate in a workshop at Bethesda, Maryland, 19 to 20 November 1973, to review the mechanisms of connective tissue degradation and to identify promising areas for future research. Workshop participants discussed the structure and degradation of proteoglycans, collagen, and elastin, and considered some of the relationships between inflammatory disease and connective tissue degradation.

Harold Fullmer (University of Alabama, Birmingham) opened the workshop by describing the pathological events that result in destruction of the connective tissue supporting structures in periodontal disease. He suggested that periodontal disease could possibly serve as a model for the study of the mechanism of connective tissue degradation accompanying inflammation. The various components of connective tissues were then discussed in different sessions.

Current concepts of proteoglycan structure were reviewed by Lennart Rodén (University of Alabama, Birmingham). He presented a scheme by which proteoglycans could be degraded by the action of a number of complementary exoglycosidases. He pointed out that proteolysis modifies primary biosynthetic products to yield partially degraded molecules. This is particularly evident in the case of heparin. Bryan Toole (Massachusetts General Hospital, Boston) reported that changes in the species of certain connective tissue macromolecules parallel events in differentiation. After amputation of a newt limb, the highly cellular regenerating limb bud accumulates large quantities of hyaluronic acid. Before cartilage can be synthesized the accumulated hyaluronic acid is degraded by a hyaluronidase. Toole also found that precartilage cells from chicks did not form cartilage nodules in cell culture when microgram quantities of hyaluronic acid were added to the culture medium. The inhibition of nodule formation by hyaluronic acid could be overcome by the addition of thyroxin, calcitonin, cyclic adenosine monophosphate (AMP), or glucosamine. These data suggest that proteoglycans may significantly influence cellular activity during differentiation.

John Dingle (Strangeways Laboratories, Cambridge, England) reported on the role of lysosomal proteinases in connective tissue degradation. Using a specific antiserum to cathepsin D, his group has shown that there is extracellular release of cathepsin D into cartilage matrix in experimental arthritis and human rheumatoid arthritis. The source of this enzyme seems to be the juxta-articular inflammatory tissue. Other enzymes that appear to be involved in cartilage proteoglycan degradation are cathepsins B and F. The latter is a newly discovered proteinase capable of degrading cartilage matrix. He suggested that matrix degradation was a complex phenomenon comprised of an extracellular phase instigated by collagenase, cathepsin B, cathepsin D, and possibly cathepsin F. This is followed by phagocytosis of connective tissue components and further degradation of matrix components within the lysosomal system of the phagocytic cells.

Discussions of the degradation of collagen centered around collagenase, an enzyme that specifically cleaves the collagen molecule three-quarters of the way from the NH_2-terminal end. Edward D. Harris, Jr. (Dartmouth Medical School, Hanover, New Hampshire), demonstrated that such a cleavage is facilitated by the native helical conformation of the molecule since denatured collagen or gelatin was only slowly cleaved by a highly purified collagenase. The purified enzyme was derived from an ascites cell carcinoma growing in muscle in rabbits. Collagenase cleavage is highly specific because incubation of collagenase with cyanogen bromide collagen peptides of similar composition, which contain the glycylisoleucyl sequence, α1 CB7 and α1 CB8, results only in cleavage of that peptide (α1 CB7) which contains the site of cleavage in the intact molecule. A possible explanation for this phenomenon, suggested by Jerome Gross (Massachusetts General Hospital, Boston), was that the peptide bond cleaved by collagenase is characterized by adjacent bulky side chains and few stable triplets. The other sequences around the glycylisoleucyl sequence in the chain have either low residue volumes or higher quantities of stable triplets. However, it is still plausible that a unique sequence involving an undetermined number of residues in the region of cleavage is a key factor. Gross also reported that highly purified tadpole collagenase was capable of cleaving cartilage collagen, $[\alpha 1\ (II)]_3$. David Woolley (University of Manchester, England) also reported that highly purified skin collagenase was capable of degrading cartilage collagen and Harris made similar observations with rheumatoid synovial collagenase. Both investigators found that cartilage collagen is significantly more resistant to collagenase action than skin collagen.

Mary Burleigh (Strangeways Laboratories, Cambridge, England) described the isolation of a specific collagenase from rabbit synovial cell cultures. This enzyme was secreted into serum-free medium of synovial cells throughout their life-span in culture, but was not found in extracts of the cells themselves.

Arthur Eisen (Washington University Medical School, St. Louis), using an antibody against human skin collagenase, has localized the enzyme to the papillary dermis of human skin and to human skin fibroblasts grown in monolayer culture. He has also used the antibody to develop an exceedingly sensitive immunoassay for human and rat collagenases. A zymogen of collagenase, found in tadpole tissue, was discussed by Elvin Harper (University of California, San Diego). The zymogen did not cleave collagen but reacted with an antibody to tadpole collagenase. Incubation of the zymogen with a two-component system found in the medium of cultured tadpole tails resulted in activation of the enzyme and collagenolysis. Burleigh also demonstrated that both collagen in solution and insoluble tendon collagen were degraded by cathepsin B1, a lysosomal thiol proteinase, at acid *p*H.

Karl Piez (National Institute of Dental Research, Bethesda, Maryland) discussed the structure and function of

elastin. He pointed out that nuclear magnetic resonance studies show that the polymer chains of this macromolecule are in rapid and continuous motion, like the chains in rubber, suggesting a common mechanism of elasticity. Elastin appears to be degraded by a family of elastases. Three different enzymes capable of degrading synthetic elastase substrates and insoluble elastin have been purified from human granulocytes by Aaron Janoff (Health Science Center, Stony Brook, New York). Ordinarily these enzymes appear to degrade bacterial constituents after phagocytosis, but are capable of damaging connective tissue elastin if they leak out of the cells. Kjell Ohlsson (University of Malmo, Sweden) also found and has successfully separated three elastases, with a molecular weight of 30,000, from extracts of human granulocytes. These enzymes are inhibited by both $\alpha 1$ antitrypsin and $\alpha 2$ macroglobulin. Ohlsson was also able to isolate from granulocytes two species of collagenase that were inhibited by these serum proteins. Burleigh reported that all endopeptidases tested, from all four proteinase classes, are inhibited by $\alpha 2$ macroglobulin on a mole for mole basis. She suggested that proteinases cleave a peptide bond in a sensitive region of the macroglobulin and that this results in a conformational change in the $\alpha 2$ macroglobulin molecule that traps the enzyme irreversibly. Access of substrates to the active site of the enzyme becomes sterically hindered, causing inhibition that is most pronounced with large substrate molecules.

Neutral proteinase extracted from rabbit skin appears to be capable of degrading structural connective tissue proteins. Gerald Lazarus (Albert Einstein College of Medicine, New York) reported that injection of the partially purified proteinase into rabbit skin resulted in a wheal and flare within 15 minutes and migration of granulocytes within 20 hours. He suggested that this proteinase might be released during tissue injury and could instigate the inflammatory cascade. Gerald Weissmann (New York University Medical Center, New York) described experiments in which secretion of lysosomal proteinases was inhibited by cyclic AMP. Secretion of lysosomal proteinases was induced by cyclic guanosine monophosphate, cholinergic agents, and aggregation of microtubules.

Irma Gigli (Harvard Medical School, Boston) discussed the pathology of activation of the complement system and its implications in connective tissue degradation through the elicitation of acute inflammatory responses. The role of delayed hypersensitivity in connective tissue degradation in general and periodontal disease specifically was evaluated by Stephen Mergenhagen (National Institute of Dental Research, Bethesda, Maryland). Lymphocytes from patients with periodontal disease undergo blast transformation when they are incubated with dental plaque antigens. Such a phenomenon results in the elaboration of a lymphotoxin, which kills gingival fibroblasts and also results in the production of an osteoclast activating factor. Transformed lymphocytes also appear to elaborate a material capable of inducing macrophages to produce a specific collagenase. Thus, a biological basis is emerging which could account for the connective tissue destruction seen in chronic periodontal disease.

At the conclusion of the workshop, promising areas for future research were identified. First, the structure of connective tissue substrates and factors that alter their susceptibility to degradative enzymes should be characterized. Second, the enzymes responsible for connective tissue degradation should be precisely identified, and the cellular control mechanisms governing synthesis, secretion, and action of these enzymes should be elucidated. Third, the role of connective tissue enzymes in normal turnover of connective tissue macromolecules and the balance between synthesis and degradation should be defined. Fourth, the role of disease states in altering enzyme activity should be probed. Such studies may offer a means by which connective tissue degradation could be manipulated in order to prevent destruction of connective tissue structures.

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Insecticides for the Future: Needs and Prospects

"Insecticides for the future: needs and prospects" was the theme of an international conference held 22 to 27 April 1974 at the Bellagio (Italy) Conference Center. Insecticides now are, and in all likelihood will continue to provide, the backbone of applied pest control in the developed countries of the world. Their use at present is somewhat limited in most of the developing countries, but in a real sense the future shape of agriculture (W. R. Furtick, Food and Agriculture Organization) and public health (J. W. Wright, World Health Organization) in the entire world will depend largely on the availability of the proper kinds of insecticides in adequate quantities. According to R. F. Smith (University of California, Berkeley) the pesticide "crisis" that many of the developing countries face at present may more properly be defined in terms of shortages of these compounds than their excessive use or misuse.

Current trends indicate that the organophosphorus (E. Y. Spencer, London, Ontario, Canada) and carbamate (T. R. Fukuto, University of California, Riverside) insecticides will continue to bear the burden as pest control agents in the immediate future. A great need remains for such compounds with more selective properties, both between species of insects and between insects and mammals (G. T. Brooks, University of Sussex, England). Where insect species specificity is involved, however, the already high—and accelerating—cost of evaluating and registering insecticide compounds looms as a serious obstacle to the desired specificity.

Long-range planning to overcome insect control problems must include the development of strategies based on exploiting those aspects of insect biochemistry and behavior for which there exists no counterpart in vertebrates. Particularly promising avenues worth exploring include insect endocrinology, with special emphasis on (i) juvenile hormone antagonists (W. S. Bowers, New York State Agricultural Experiment Station, Geneva), (ii) peculiar characteristics of insect cuticle and a greater understanding of the ways insecticides penetrate this barrier (M. Locke, University of Western Ontario, Canada), (iii) a deeper knowledge of insect nerve membranes and transmitter substances in insect synapses and neuromuscular junctions (T. Narahashi, Duke Univer-