resides in the 536 nucleotides and the polyadenylate stretch that are present on the 3′ side of the coding region. At present we cannot rule out the possibility that a small noncrystallin polypeptide is coded within this region. Although the length of this 3′ region is large in comparison with numerous other mRNA's, such as the 92-nucleotide untranslated region of rabbit β-globin mRNA (*25*), it is not unprecedented among eukaryotic mRNA's. For example, the 3′ untranslated sequence of chicken ovalbumin mRNA consists of 634 nucleotides (*26*). Also, the length of the 3′ untranslated region for the dihydrofolate reductase mRNA in the mouse ranges from about 80 nucleotides to about 930 nucleotides in the four different RNA's found for this enzyme (*27*). The size of the polypeptide encoded by the dihydrofolate reductase mRNA's is approximately the same as αA-crystallin. The function of the sequences on the 3′ side of the coding region is not known yet for any mRNA. Possibly, comparisons of the 3′ sequences of the 14*S* mRNA for α-crystallin from different organisms will reveal stretches of conserved nucleotides and provide insight as to their functional significance.

Note added in proof: After submission of this report, the sequence of a rat αA-crystallin cDNA was reported (*30*). The findings were similar to those reported here. Comparison of the 3′ noncoding regions of the mouse and rat αA-crystallin cDNA's show that 24 insertion-deletion and 32 base change events have occurred during the evolutionary divergence of these organisms. There are 18 synonymous base differences within the encoding region.

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Cardiac Catabolic Factors: The Degradation of Heart Valve Intercellular Matrix

Abstract. *Cultures of porcine heart valves and aorta secrete a factor that stimulates the degradation of cartilage matrix in a fashion similar to that displayed by synovial catabolin. The heart valve factor also induces the release of chondroitin sulfate and hydroxyproline from isolated heart valve cultures. The present observations support the hypothesis that tissues producing catabolic factors (catabolins) may well be responsive to them and that these messengers may play a role in the cellular regulation of the degradation of intercellular macromolecules.*

Our present understanding of the mechanisms associated with the degradation of intercellular macromolecules stems largely from observations on cartilage matrix breakdown. Until recently the local invasion of the articular cartilage by synovial pannus was believed to be responsible for the degradation of proteoglycan and collagen that constitute the major structural components of the matrix. However, Fell and Jubb (*1*) and somewhat later Steinberg's group (*2*) demonstrated that normal as well as rheumatoid synovium secreted a diffusible product which induced chondrocytes to destroy their own matrix. Dingle and co-workers (*3–5*) have isolated and partially characterized a small acidic protein (catabolin) from porcine synovium which also stimulates viable chondrocytes to rapidly degrade their matrix. Although it is apparent that synovial catabolin initiates the breakdown of cartilage matrix in vitro, it is not known whether there is a more fundamental role for such factors in regulating the content and distribution of intercellular macromolecules in other tissues. It is possible that autologous and heterologous classes of catabolin-like factors mediate the intercellular turnover of proteoglycan and collagen, and that the interaction of "catabolins" with other factors that promote the synthesis of matrix components control the composition of connective tissue elements under altering physiolog-

Table 1. Tissues and cells producing and responding to catabolin. N.D., not determined.

Tissues or cells	Production	Response
Synovium (human)	+	N.D.
Synovium (pig)	+	+
Synovium (rabbit)	+	N.D.
Cartilage (human)	N.D.	+
Cartilage (pig)	N.D.	+
Cartilage (rabbit)	N.D.	+
Heart valve (pig)	+	+
Blood vessels (pig)	+	+
Placenta	+	N.D.
Kidney	+	N.D.
Fibroblasts (human synovial)	+	N.D.
Fibroblasts (pig synovial)	+	N.D.
Blood vessel (smooth muscle cells)	+	N.D.
Blood vessel (endothelium cells)	+	N.D.
Activated monocytes	+	+

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ical and pathological states (*6*). However, if this were the case the release of putative catabolic factors from a specific tissue would have to initiate the depletion of or enhance the turnover of the intercellular matrix of that same tissue. We have investigated this hypothesis using vascular tissue as the test system.

Heart valves, including the chordae tendinae, the proximal 2 cm of the aorta, and the papillary muscles, were removed from hearts of freshly slaughtered pigs and minced. The minced tissue was cultured in Dulbecco's modified Eagle's medium (DME) at 37°C in an atmosphere of 5 percent CO_2 and 95 percent air (*3*). The medium was changed every other day for up to 14 days, with each portion being diluted 1:1 with DME plus 10 percent fetal calf serum. This medium was then tested for its catabolic activity on bovine nasal cartilage disks (1 by 3 mm) or on portions of heart valve (50 mm^2). After culturing the tissue for up to 2 weeks we measured the chondroitin sulfate and hydroxyproline content in portions of the medium and in a papain digest of the cartilage and heart valves (*7, 8*).

Cultivation of bovine nasal disks in the presence of medium obtained from cultured heart valve and aorta induced the degradation of cartilage matrix, whereas medium from papillary muscle cultures elicited little or no release of chondroitin sulfate (Fig. 1). This response required the presence of live cartilage; dead cartilage failed to respond to either catabolin or the heart valve factors. The kinetics of chondroitin sulfate liberation stimulated by these cardiac factors closely resembled the kinetics of chondroitin sulfate release in response to partially purified synovial catabolin (Fig. 1). After 2 weeks in vitro, disks exposed to catabolin had released approximately 85 percent of the chondroitin sulfate, whereas those exposed to the heart factors showed a 65 to 80 percent breakdown of cartilage matrix (Fig. 1).

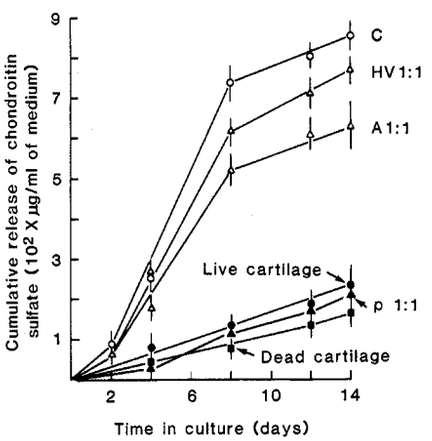

Fig. 1. Kinetics of chondroitin sulfate release from bovine nasal cartilage in response to partially purified synovial catabolin (*C*) and catabolic factors from pig hearts. Bovine nasal disks were exposed for 2 weeks to catabolin (20 µg/ml) or 1:1 dilutions of medium from 4-day cultures of heart valve (*HV*), aortic (*A*), or papillary (*P*) muscle. At specified intervals the medium was digested with papain and the chondroitin sulfate was determined by means of dimethylmethylene blue reagent. After 2 weeks the remaining disks were also digested and the results were expressed as micrograms of chondroitin sulfate released per milliliter of medium (mean ± standard error). Live and dead cartilage disks were used as controls to demonstrate the degree of spontaneous release of matrix which occurs over the course of the experiment. All assays were conducted in quintuplicate.

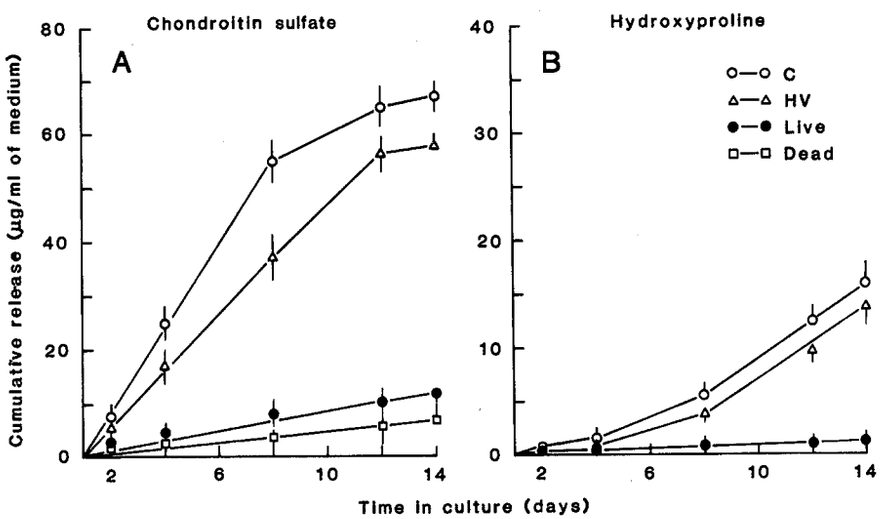

Fig. 2. The kinetics of (A) chondroitin sulfate and (B) hydroxyproline release from heart valves (50 mm^2) cultured in the presence of porcine synovial catabolin (*C*) or a 1:1 dilution of heart valve (*HV*) medium. Chondroitin sulfate was measured as outlined in Fig. 1. Hydroxyproline was determined in portions of the papain digests of media and heart valves by means of Stegemann's method as modified by Burleigh *et al.* (*8*). Controls consisted of cultures that were not supplemented or tissue that had been killed by three repeated freeze-thaw cycles. Chondroitin sulfate and hydroxyproline content was expressed as micrograms of the glycosaminoglycan or amino acid acid per milliliter of medium (mean ± standard error). All assays were conducted in quintuplicate.

The medium derived from cultured heart valves was then itself exposed to freshly explanted valves, and chondroitin sulfate and hydroxyproline were measured to determine whether autologous media would induce the degradation of valvular extracellular matrix. Although heart valves contain far less proteoglycan than bovine nasal cartilage (10 percent of that in a cartilage disk), synovial catabolin and the heart valve medium both stimulated the release of chondroitin sulfate with almost identical kinetics (Fig. 2A). Maximum release appeared to occur after exposure to the factors for 12 days. Control cultures of live and dead tissue spontaneously released very little proteoglycan during this intervening period. In contrast, the degradation of collagen proceeded much more slowly with little release of hydroxyproline being apparent until day 8 of culture. Even after 2 weeks of exposure to either catabolin or the heart valve factor only 30 to 40 percent of the collagen appeared degradable (Fig. 2B).

These experiments indicate that cultured heart valves and aorta, but not papillary muscle, may be added to the list of cells and tissues (Table 1) that produce a factor that stimulates the breakdown of proteoglycan and collagen in bovine nasal disks. Since the kinetics of chondroitin sulfate release closely parallel those produced by synovial catabolin, it is possible that these cardiac factors are similar or identical to synovial catabolin (*4*).

The heart valve factor acts on the tissue of its origin and generates the degradation of its own intercellular matrix. It is tempting to suggest that the regulated secretion of these factors may interact with those controlling synthesis, thereby modulating the turnover of matrix macromolecules, and that only during severe pathological circumstances do they provoke tissue injury. One such pathological insult, termed "floppy valve," is characterized by a progressive depletion of extracellular matrix from diseased mitral valves (*9*). Although the etiology of the disease is unknown, the depletion of collagen, elastin, and glycosaminoglycans is extensive and is accompanied by a mild cellular hyperplasia

(*10*). Since no mononuclear inflammatory cells have been observed in this lesion, it has been proposed that resident fibroblasts are responsible for the matrix destruction. It is conceivable that catabolic factors of the variety secreted from porcine mitral and aortic valves mediate such tissue injury.

The present observations imply a possible role for such factors during development of degenerative disease; however, their mode of action implies that they might function in the normal turnover of extracellular macromolecules as well. Tissue catabolins may also be active during wound healing. Since human and porcine synovium fail to produce catabolin in the presence of hydrocortisone (*11*), the poor wound healing that frequently attends corticosteroid therapy may well reflect an inhibition of turnover and repair processes that require these factors. Therefore, the hypothesis that catabolins may have a more general role in cell-tissue interactions may be pertinent not only to our understanding of the mechanisms that modify tissue integrity but also to the pharmacological control of tissue damage in catabolic diseases.

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Anti-Inflammatory Steroids Without Pituitary-Adrenal Suppression

Abstract. *When two new steroids, methyl prednisolonate and methyl 20-dihydroprednisolonate, were applied locally their anti-inflammatory activities were nearly equivalent to those of the parent compound prednisolone in the cotton pellet granuloma bioassay. However, when these two derivatives were administered systemically, their anti-inflammatory activities were weaker than those of the parent compound. Furthermore, unlike the parent compound, these new anti-inflammatory steroids did not suppress pituitary-adrenal function or cause liver glycogen depletion in rats.*

Although the beneficial effects of natural and semisynthetic corticosteroids in the treatment of inflammatory and allergic conditions have been appreciated for over 30 years, complications arising from steroid therapy have imposed limitations on the clinical use of this class of drugs (*1*). The shortcomings are largely inherent in the nature of corticosteroids themselves; not only do glucocorticosteroids possess multiple biological activities, but the structural requirements for various activities appear to be overlapping and inseparable. If the actions of corticosteroids could be localized, many of the complications could be eliminated. Although methods for the local administration of steroids have been devised (*2*), complications associated with local steroid treatment for psoriatic, rheumatologic, eczematous, asthmatic, and ophthalmic patients have been reported (*3*). This situation calls for new approaches in developing anti-inflammatory steroids that are devoid of toxicities.

In developing a new concept, we were guided by several considerations: (i) corticosteroid pharmacotherapy appears to offer an abundance of agents, but no truly safe drug; (ii) systemic effects of steroids are unnecessary complications which accompany treatment of many inflammatory conditions; (iii) an intact ketol side chain is not an absolute requirement for the anti-inflammatory activity of corticosteroids (*4*, *4a*, *5*); and (iv) steroid acid esters with intact ring structures corresponding to the known potent glucocorticoids retain anti-inflammatory activity but upon entry into the circulatory system from the site of administration are hydrolyzed to steroid acids that are inactive and readily excreted (*6*).

We now report that ester derivatives of steroid-21-oic acids, applied locally, possess anti-inflammatory activity equivalent to the parent compound but do not suppress adrenal function or liver glycogen content in rats.

The anti-inflammatory activities of methyl prednisolonate (*7*), methyl 20-dihydroprednisolonate, and 20-dihydroprednisolonic acid (*8*) were evaluated in the cotton pellet granuloma bioassay in rats (*9*). Thymolytic, liver glycogen depository, and pituitary-adrenal (PA) suppressive effects were monitored. When the rats were under mild anesthesia we implanted two cotton pellets (35 ± 1 mg each) subcutaneously, one in each axilla. The local effects of the steroids on granuloma formation were determined by injection of the compound into the cotton pellet before implantation; the systemic effects were evaluated by giving daily intramuscular injections of the compounds after pellet implantation. Seven days after implantation the rats were killed and granuloma, adrenal, thymic, and body weights were measured. Blood samples were analyzed for adrenocorticotropin (ACTH) (*10*) and corticosterone (*11*) and livers were analyzed for glycogen content (*12*).

Prednisolone caused a significant decrease in all the values measured in control rats. In contrast, the new steroids methyl prednisolonate and methyl 20-dihydroprednisolonate, when they were administered locally, selectively suppressed the weights of granulomas and thymus glands but did not alter adrenal weights, plasma ACTH, plasma corticosterone, or liver glycogen (Fig. 1A). In this study high doses of steroids were administered deliberately in order to detect any possible systemic toxicities. At the dose level of 2.5 mg per pellet, methyl prednisolonate and methyl 20-dihydroprednisolonate decreased granuloma formation by 56.9 percent and 58.6 percent, respectively. These values are comparable to the 58.3 percent granuloma inhibition obtained with the same dose of the parent compound prednisolone, and suggest that some degree of freedom is available for modifying the ketol side chain of corticosteroids without losing anti-inflammatory activity. This is supported by the anti-inflammatory activity reported for the fluocortolonate esters (*4a*, *13*). However, with doses of 5 mg per pellet, prednisolone exhibited a higher inhibitory effect on granuloma formation (70.29 percent) than either methyl prednisolonate (55.6

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