Meetings

Catabolism of Collagen

Collagen, the major structural protein of vertebrates, is considered to be one of the most inert proteins in terms of the normal turnover and catabolic processes. In certain pathological conditions and a few normal ones, such as the remodeling of bone and postpartum uterine changes, collagen resorption can occur rapidly. The nature of the stimulus for collagen resorption, the collagenolytic system involved, the effects of cross-linking on the susceptibility to breakdown, and the fate of the degraded fragments are some of the important problems facing investigators. A workshop on collagen catabolism, held at Western Reserve University School of Medicine, 26–27 February 1965, provided an opportunity for researchers in this field to discuss these problems and to present the results of their investigations.

A discussion of crosslinkages was presented by A. Veis (with J. Anesey, Northwestern University) who investigated gelatins obtained by the denaturation of mature rat-tail tendon (RTT) collagen and by the isionic solubilization, at 60°C, of purified mature bovine corium collagen (IE). The subunit chain components were eluted from the carboxymethyl cellulose columns in the order of $\alpha 1$, β_{11}, γ_{111}, γ_{112}, β_{12}, $\alpha 2$, β_{22}, γ_{122}, γ_{222}, δ. The chromatographically distinct fractions were then examined by sedimentation analysis, disc electrophoresis, renaturability, and amino acid analysis. From these data it was concluded that the two tissues contained qualitatively identical components. Although intermolecular species predominated in corium they were also present in substantial quantity in tendon. All components except $\alpha 1$, β_{11}, β_{12}, $\alpha 2$, and γ_{112} were presumed to be of intermolecular origin. The enhancement of intermolecular bonding between $\alpha 1$ subunit chains was considered to be a distinct feature of the maturation of collagen. Also, inter- and intramolecular polymerization involve the same functional groups acting competitively.

W. van B. Robertson (with Kirrane, Stanford University) reported on some studies of tyrosylated collagen containing 1.6 to 8.0 percent tyrosine. Solubility in salt at *p*H 7.4 decreased with increasing tyrosine content, but all preparations were readily soluble in 0.1*N* acetic acid. The fraction precipitable at 45°C with trichloroacetic acid increased with increasing tyrosine content; the rate of gelation at 38°C increased with increasing tyrosine concentration. Optical rotation of native and denatured collagen was independent of tyrosine concentration. In contrast to unmodified tropocollagen which did not stimulate antibodies in rabbits, collagen containing 1.6 to 2.0 percent tyrosine stimulated production of antibodies which cross-reacted strongly with rat skin tropocollagen.

In response to necrotic injury and the administration of various hormones such as cortisol, thyroxin or prolactin, abrupt losses of dermal collagen (particularly of insoluble collagen) were observed in rats (J. C. Houck, Georgetown University). Concomitantly a nonlysosomal protease is released into the extracellular, extra-fibrillar compartment. This protease is maximally active at neutral *p*H and is inhibited by soybean trypsin inhibitor and salicylate. It resembles another protease previously found in epidermis. Paralleling the release of this protease, amounts of soluble RNA and activated collagenase also increased. This collagenase can cause the dissolution of insoluble collagen into two products, one diffusing away from the tissue and the other newly soluble in dilute acid. G. Nichols (Harvard) described another collagenolytic system. Relatively pure and undenatured, C^{14}-labeled, collagen substrate was prepared by incubation of rat bone with C^{14}-labeled proline in vitro. After incubation, decalcification and extensive extraction were used to remove cell proteins. Collagenase activity of preparations was estimated in terms of the amount of collagen substrate (calculated from radioactive data) rendered ultrafilterable after incubation at 37°C for 40 minutes. Collagenolytic activity was readily demonstrated by this method in a particulate fraction of homogenates of cells isolated intact from bone. Evidence suggested that this fraction contained lysosomes or similar bodies which in turn contained the enzyme. The relation of this collagenase activity to bone resorption was indicated by the facts that it was virtually confined to bone cells, and that the total activity, but not the intracellular distribution, was increased following parathyroid extract. Finally, the importance of this collagenase activity in the development of the experimental osteoporosis, induced by heparin, was indicated by experiments in which heparin in vitro had direct lytic effects on cell particle membranes—an effect which was thought to be closely related to the marked increase in bone cell collagenolytic activity induced by chronic heparin therapy in vivo. As described by M. Tanzer (with J. Gross, Harvard) a highly specific collagenolytic enzyme was isolated from tadpole skin, and then purified 300-fold. The enzyme attacked native collagen at a specific locus, cleaving the three chains of tropocollagen approximately one quarter the distance from the carboxyl terminus and yielding short and long intact helical segments. Although the native conformation of these pieces seems to be retained, their thermal denaturation temperature is lowered, thus suggesting that the collagenase attack may trigger further degradation of the segments by relatively nonspecific proteases.

Tanzer also described studies of the resorbing fin of metamorphosing tadpoles. Using isotope techniques, he showed that active collagen synthesis occurred during resorption, and old, nonradioactive collagen fibrils were selectively removed. Simultaneously, there was a continuous deposition of new radioactive fibrils thus providing the structural integrity of the diminishing tail basement membrane. Further evidence of selective, regional collagen catabolism was obtained by finding that prelabeled fins produce a continuously changing specific activity of collagen breakdown products as the fins resorbed in tissue culture.

Studies in vivo and in vitro by

G. Weissmann (New York University) showed that enzymes released from lysosomes can degrade the protein-polysaccharides of cartilage. Both in vivo and in vitro, cortisone and its analogues retard the release of enzymes from lysosomes; other steroids and membrane active agents, such as vitamin A, accelerate release. In amphibian metamorphosis, not only the metachromatic material of amphibian tail is resorbed under the influence of vitamin A, but also collagen is lost. This effect can be significantly retarded by treatment of premetamorphotic larvae with cortisone or chloroquine. Weissmann described studies during which he used synthetic lipid spherulites. The membranes of such spherulites resemble those of lysosomes or erythrocytes in their response to steroids or other agents, and suggest that effects of steroids on the permeability of cells and organelles may be explained by their direct interaction with surface lipid, independent of sugars, proteins, or cell metabolism. It was further suggested that, if collagenase of mammalian cells is indeed a lysosomal enzyme, its release could be governed by steroid hormones.

In order to investigate the possible role of lysosomes in uterine involution, J. F. Woessner (Howard Hughes Institute) assayed for a variety of uterine acid hydrolases which are normally found in the lysosomal fraction of rat liver. He found that cathepsin, β-galactosidase, and acid phosphatase increase in concentration by 240 to 400 percent during involution. Studies of the subcellular distribution of these enzymes were inconclusive because of problems encountered in homogenizing the uteri. However, the proportion of the enzymes in the lysosomal fraction appeared to increase during involution. The hypothesis is presented that the major feature in the breakdown of uterine collagen is phagocytosis by macrophages and possibly other endocytic cells, followed by intracellular digestion involving lysosomal enzymes. The earliest step of the breakdown may involve a secreted collagenase. Attempts to show a mammalian collagenase in homogenates, prepared by various methods from various species of uteri, have been unsuccessful. However, collagen is digested in vitro at *p*H 3 to 4 by a uterine cathepsin which was purified 1000-fold. It has a specificity identical to that of cathepsin D.

From two systems, the chick embryo and guinea pig skin, D. S. Jackson (Oregon University) presented evidence that free hydroxyproline and peptide-bound hydroxyproline is derived, in part, from breakdown of newly synthesized collagen. In a study of wound-healing in the colon, he found that after the wound is made collagen degradation takes place at a distance from the wound along the colon. Almost simultaneously there is a rapid increase in the rate of collagen synthesis in the same area, concomitant with the formation of collagen in the wound itself.

D. J. Prockop (University of Pennsylvania) reported on his studies of hydroxyproline excretion. Proline-C^{14} was injected into young rats and the specific activity of the hydroxyproline-C^{14} excreted in urine was compared with the specific activity of the hydroxyproline-C^{14} in soluble and insoluble collagen. Fifteen hours after the proline-C^{14} injection, the specific activity of the urine hydroxyproline-C^{14} was comparable to the specific activity of hydroxyproline-C^{14} in soluble collagen. The results were consistent with earlier observations which suggested that part of the hydroxyproline excreted in urine originates from the degradation of early forms of collagen such as soluble collagen. Four weeks after the administration of the isotope, the specific activity of urine hydroxyproline-C^{14} was considerably greater than the specific activity of hydroxyproline in soluble collagen, but somewhat less than the specific activity of the hydroxyproline in insoluble collagen. Calculations performed with the data obtained from two rats indicated that between 60 and 75 percent of the hydroxyproline peptide excreted in urine comes from the degradation of insoluble collagen and that only 5 to 10 percent of the hydroxyproline released during the degradation of insoluble collagen was excreted in urine.

L. Klein (Western Reserve) reported that concentrations of hydroxyproline in urine did not reflect bone resorption in rats treated with large doses of parathyroid extract and did not reflect collagen resorption during the post-partum involution of the human uterus. The in vivo catabolism of collagen was studied by administering C^{14}-gelatin parenterally to rats. Of the catabolized C^{14}-hydroxyproline, 20 to 30 percent appeared as peptides in the urine. In preliminary experiments C^{14}-hydroxyproline peptides excreted by chronically labeled rats were given parenterally to unlabeled animals. Almost quantitative (70 to 90 percent) urinary excretion of C^{14}-hydroxyproline was found, indicating that urinary hydroxyproline peptides were not catabolized. These data indicate that a relatively constant proportion of the gelatin catabolized is excreted in the urine, which casts doubt on the catabolic nature of collagen resorption.

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Forthcoming Events

June

28–30. **Electromagnetic Scattering**, conf., Univ. of Massachusetts, Amherst. (R. S. Stein, Polymer Research Inst., Univ. of Massachusetts, Amherst)

29. National Council of **Teachers of Mathematics**, New York, N.Y. (J. D. Gates, NCTM, 1201 16th St., NW, Washington, D.C. 20036)

29–1. **Mutant Mice with Neurological Diseases**, conf., Jackson Laboratory, Bar Harbor, Maine. (J. L. Fuller, Jackson Laboratory, Bar Harbor)

29–2. **Data Processing Management** Assoc., intern. conf., Philadelphia, Pa. (Conference Registrar, Data Processing Management Assoc., P. O. Box 1079, Philadelphia 19105)

30–2. **Microwave Applications of Semiconductors**, symp., University College, London, England. (Symposium Secretary, Inst. of Electronic and Radio Engineers, 8-9 Bedford Square, London, W.C.1)

30–3. National Soc. of **Professional Engineers**, annual, Albuquerque, N.M. (NSPE, 2029 K St., NW, Washington, D.C.)

July

1–4. **Astronomical** League, Miami, Fla. (A. P. Smith, Jr., 1601 S.W. 10th St., Miami)

1–10. General **Relativity and Gravitation**, intern. conf., London, England. (H. Bondi, Dept. of Mathematics, King's College, London, W.C.2)

1–1 August. **Theoretical Biology and Theoretical Biophysics**, Colorado State Univ. colloquium, Fort Collins. (H. J. Morowitz, Dept. of Molecular Biology and Biophysics, Box 2166, Yale Station, New Haven, Conn.)