TERMINATION REPORT

NASA Contract No. NGR 09-134-001

Investigation of the effect of stress on the chemistry, metabolism, and biophysics of collagen

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Principal Investigator: John C. Houck, Ph.D.

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The question to be addressed by the work supported by this contract (NASA Contract No. NGR 09-134-001) was to determine precisely the effect of stress upon the chemistry of the connective tissue of the rat. The results of this study took longer to publish than the duration of the contract itself, but these results are summarized in two papers which acknowledge NASA support, one in Advances of Biology of Skin, Vol. 10, The Dermis, edited by Montagna, Bentley, and Dobson, published by Appleton-Century-Crofts, New York, Chapter 4, page 49, "The Catabolism of Cutaneous Collagen," (1970); the other in Advances in Enzyme Regulation, Vol. 8, edited by George Weber, published by Pergamon Press, New York, page 269, "Control of Cutaneous Collagenolysis," (1970).

Metabolically, insoluble cutaneous collagen has a biological half-life in the rat of about one year. Despite this low rate of turnover, within a day after the administration of cortisol (stress hormone), a significant amount of this insoluble collagen disappeared from the skin. Since this loss approximates 500 mg for the skin of a 200 gram rat, or about 25% of the total cutaneous collagen of this animal, and since the total turnover for all tissue collagen including bone and tendon has been estimated to be only 50 mg per rat per day, obviously the inhibition of collagen anabolism by steroid could not explain the ten-fold greater loss of collagen from the skin of animals receiving steroid.

Since collagen is entirely extracellular and since steroids do not lead to the infiltration of blood cells into the connective tissue,

the rapid loss of skin collagen resulting from steroid administration *in vivo* must be mediated by the release of a collagenolytic enzyme into the extracellular space of the tissue.

We have isolated and purified a collagenolytic enzyme which preexists as an inhibitor-collagenase complex in the extracellular, extrafibular space of the connective tissue of the skin. This collagenase can be activated by limited proteolysis of the inhibitor, releasing free collagenase from the complex. This process takes place during necrosis as the consequence of the discharge of lysosomal proteases from infiltrating white blood cells into the wound space.

This same collagenase has been demonstrated to be induced via the derepression of an operon in diploid human fibroblasts *in vitro* by any steroid containing a β-11 OH group. As a consequence of the administration of cortisol to cultures of diploid human fibroblasts in the absence of usual serum supplementation of the medium (serum contains an inhibitor of collagenase activity), there is released into the medium a significant amount of collagenolytic enzyme similar in properties to that described previously by us for the preexisting collagenase of the dermis. This enzyme activity is not released from fibroblasts in culture when the cells are pretreated with actinomycin D or with cyclohexamide. This indicates that the synthesis of peptide bonds and of m-RNA is required for enzymatic activity to appear. The collagenase has been isolated and purified from the supernatant medium via ammonium sulfate fractionation, Sephadex column chromatography, and isoelectric focusing. The enzyme has an isoelectric point of pH 5.2 and a maximal activity on collagen

at pH 5.5. The collagenolytic activity is inhibited by phosphate buffer, serum, and the proteins found in the saline extract of skin. The purified enzyme has no hydrolytic effect upon denatured hemoglobin or casein. It is also inhibited by Dilantin but not by salicylate.

The reaction products released by this collagenase from native soluble collagen are a spectrum of variously sized small molecular weight peptides. These product peptides inhibit the collagenolytic activity itself as they accumulate in concentration. These product peptides resulting from the activity of the acid collagenase by both soluble and insoluble native collagen are further degraded by peptidases which are either also induced in the fibroblast or preexist in the skin itself. The amino acids released from the product peptides of this type of collagenolysis, including hydroxyproline, are quickly taken up by the liver and, to judge from the isotope data, are converted into liver glycogen.

It is interesting to note that the most effective amino acids for generating glycogen synthesis in rat liver in vivo are hydroxyproline, proline, glycine, and alanine; these four amino acids constitute approximately three-quarters of the amino acids found in collagen. The least effective amino acids for generating liver glycogen synthesis in vivo are the aromatic amino acids and the sulfhydral-containing amino acids; essentially none of which are contained in the structure of collagen.

Therefore it would appear that via stress hormone-induced fibroblast collagenase synthesis, the abrupt catabolism of cutaneous collagen releases small product peptides which can in turn be rapidly degraded to free

amino acids. These collagen-derived amino acids are uniquely active in effecting liver glycogen synthesis and thereby would provide a major energy source which could assist the animal in the "fight or flight" reaction to stress.

Similar collagenase induction and collagen catabolism can be demonstrated in vivo with traumatic injury, exposure to cold, and starvation. Thus, we propose that cutaneous collagen represents a hitherto unrecognized energy reserve pool which can be mobilized via stress hormone induction of collagenase activity in skin fibroblasts which is released rapidly into the extracellular space where the collagenase substrate is located.

The only pharmacologic agent that we could determine that could inhibit this cutaneous collagenolysis by stress hormone was Dilantin, as summarized in Chapter 25, page 267 of Antiepileptic Drugs, edited by Woodbury, Penry, and Schmidt, published by Raven Press, New York (1972).

The biomechanical consequences of the loss of up to 25% of insoluble collagen content of the skin remains to be determined. The relevance of this stress-hormone-induced catabolism of cutaneous collagen to the effects of null gravity on human connective tissue remains to be determined.