CONSEQUENCES OF DEFECTS IN COLLAGEN SYNTHESIS - HYDROXYLYSINE DEFICIENCY. S.M. Krane and R.S. Quinn (Boston, Massachusetts, USA)

Identification of biochemical abnormalities from subjects with heritable disorders of connective tissue has been possible within the past few years because of rapid growth in knowledge of the events in collagen metabolism. In turn, identification of the biochemical nature of such disorders has provided information about normal collagen metabolism as well as about the clinical manifestations of known biochemical defects. Hydroxylysine (Hyl) deficient collagen disease (classified by some as Ehlers-Danlos syndrome type VI) was first identified in two sisters whose clinical disorder included kyphoscoliosis, joint laxity with recurrent joint dislocations, hyperextensible skin, formation of thin scars and possibly ocular fragility (1). Dermal collagen was excessively soluble in denaturing reagents and amino acid analysis of the dermis revealed a decrease in Hyl content to $\sim$ 5% of normal whereas Lys was not reduced. Analyses of tendon and bone showed the reduction in Hyl content to be less than dermis, and cartilage approached normal despite an abnormal pattern of reducible crosslinks (2). Sonicates were obtained from fibroblasts cultured from the skin of the affected children, family members and controls and assayed at intervals after passage and attaining confluence (3). Whereas prolyl hydroxylase was normal lysyl hydroxylase in the affected children was reduced to 5-15% of levels in controls normalized to prolyl hydroxylase, lactate dehydrogenase, protein and cell number. Levels of lysyl hydroxylase in the normal sister and mother were consistent with heterozygosity. By these assays levels in fibroblasts from another patient with Hyl deficient collagenase (4) were higher than in our kindred, whereas levels in a newly discovered two year old patient presenting with muscular weakness (through Dr. P. Hanson, Albany Medical College) were reduced to the levels observed in our kindred. No inhibitor was detected. Decreased levels were seen in whole sonicates as well as 59,000 x $\bar{g}$ supernatant, 105,000 x $\bar{g}$ pellet and acetone powder of the pellets. Evidence that the low activity of enzyme in the affected children was due to abnormal enzyme protein was based on observations that the mutant lysyl hydroxylase had an apparent K_m for ascorbate of 20μM vs. normal of 4μM, greater heat instability than normal and the inability to form high molecular weight aggregates in low ionic strength buffers (5). Under culture conditions where the fibroblast sonicates in the affected children showed lysyl hydroxylase levels of 5-10%, labeled Hyl levels in collagen formed by the cells were reduced by only $\sim$ 50% however. Whereas collagen prolyl hydroxylation was depressed in all cultures without ascorbate lysyl hydroxylation was not depressed in absence of ascorbate.

In preliminary studies of dermal collagen recently obtained at time of orthopedic surgery in one child from our kindred it was found that there was heterogeneity of hydroxylation of dermal collagen, as determined by analysis of CNBr cleavage peptides. For example, no Hyl was found in α1(I)CB-VIII whereas Hyl was detected in α1(I)CB-VI and α1(I)CB-7. In contrast bone α1(I)CB-VIII contained $\sim$ 50% normal Hyl. The reasons for the Hyl heterogeneity in different tissues or within a tissue have not yet been established but could be related to variation in rates of poly-

peptide chain synthesis, alteration in lysyl hydroxylase cofactor concentrations and relative differences in rates of prolyl hydroxylation. The existence of multiple forms of lysyl hydroxylase is also a possibility.

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