AGE RELATED CHANGES IN THE REDUCIBLE CROSS-LINKS OF COLLAGEN

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1. Introduction

It is generally agreed that with increasing age the collagen fibre steadily increases in stability to external influences, e.g., thermal denaturation, swelling, solubility and enzymes (for reviews see [1–3]. All these changes could be accounted for by a gradual increase in the number of the covalent cross-linkages between the peptide chains as originally proposed by Verzar [4].

Recent studies have revealed a great deal about the nature of the cross-links in collagen. Two types of cross-link occur, *intra*molecular cross-links within the tropocollagen molecule [5] and *inter*molecular cross-links between molecules in the intact fibre [6–9]. Since *inter*molecular bonds identified were found to be labile to dilute acids and heat then to account for the decrease in solubility with age it was proposed that they were biosynthetic intermediates of the final as yet unknown stable form [10]. We have now studied the mechanism in more detail using human, bovine and rat tissues in order to compare the process in animals of widely different life spans.

2. Experimental procedure

Samples of skin, tendon, articular cartilage and bone were obtained at various ages covering the life-span of the rat, bovine and human. The samples were analysed for the presence of reducible cross-links and their precursors by amino acid analysis after reduction with tritiated sodium borohydride as previously described in detail [7]. The same amount of tissue was taken in each case and reduced under identical conditions.

The identification of the radioactive peaks as the intermolecular cross-links previously reported [6, 7], hydroxylysinonorleucine, reduced syndesine and Fr. C was confirmed by analysis against authentic samples on the amino acid autoanalyzer.

3. Results and discussion

Typical amino acid elution patterns of reduced skin, tendon and articular cartilage from 1 day old calf and 6 year old steer are shown in fig. 1. These patterns were found to be typical of the particular tissue, skin, tendon, and cartilage from the rat and human producing patterns similar to those of bovine.

In all these tissues there was a decrease in the reducible cross-links syndesine, hydroxylysinonorleucine and unknown Fr. C, with increasing age, all three being virtually absent at six years. The high proportion of Fr. C in skin at this age auggests a slower rate of maturation for skin.

An analogous series of elution patterns were also obtained for samples of skin, tendon and cartilage from humans and rats of various ages. Similar results were obtained for the human tissues except that the labile cross-links had virtually disappeared by 17–20 years. In the case of the rat, although a dramatic reduction had occurred by 6–8 months of age, a considerable amount of labile cross-links were still present even up to 3 years of age. These unusual results are probably accountable on the basis that the rat continues to lay down collagen well into its adult life.

During the disappearance of these intermediate cross-links an increase occurred in two more as yet unidentified reducible components designated Fr. A

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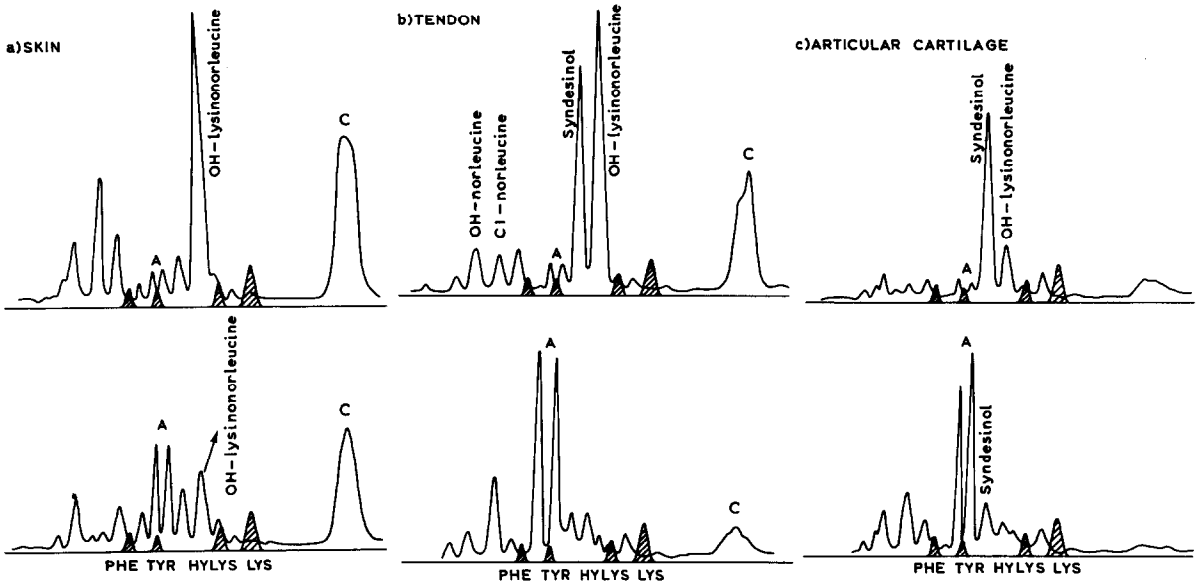

Fig. 1. Typical elution patterns of radioactive reducible components from acid hydrolysates of reduced skin, tendon and articular cartilage from young and mature tissues. The patterns shown are from 1 day old calf and 6 year old mature bovines. Similar elution patterns were obtained from young and mature rat and human tissues.

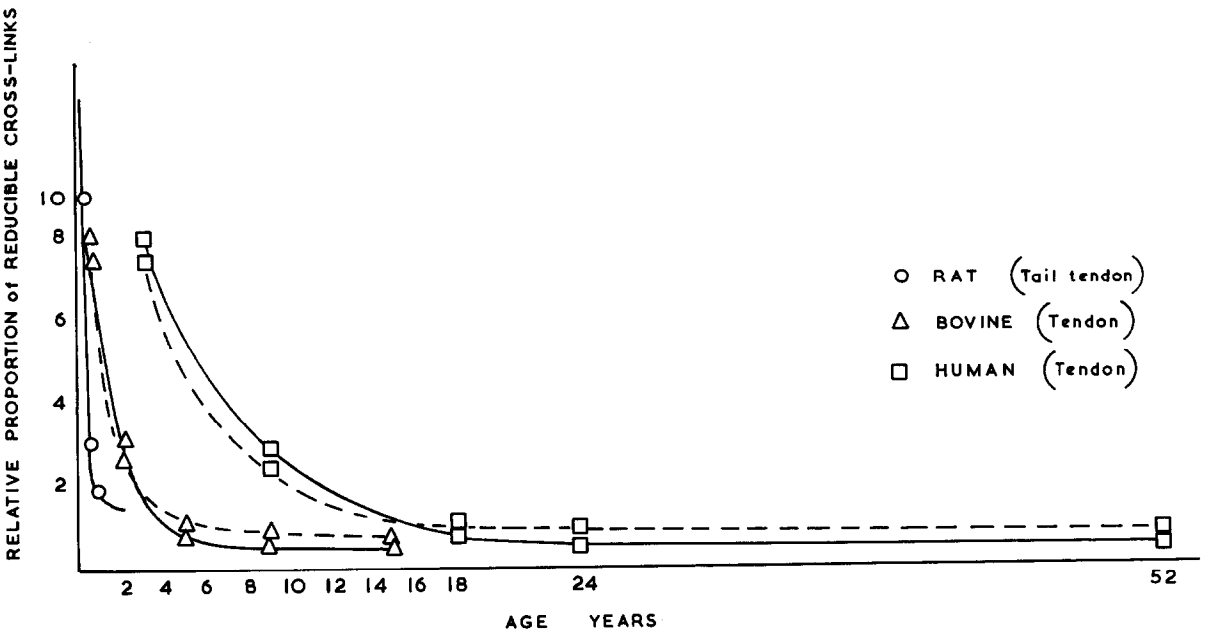

Fig. 2. Rate of change in the proportion of (i) hydroxylysinonorleucine (——) and (ii) reduced syndesine (- - - -) with increase in age for human achilles tendon, bovine achilles tendon and rat tail tendon.

(fig. 1). However, their role, if any, in the cross-linking process has not yet been elucidated. Preliminary studies have established that the component is derived from lysine.

The formation of the reducible intermolecular cross-links involves a chemical reaction of the carbonyl function of the allysine and hydroxyallysine residues with another reactive species and in view of the relative metabolic inactivity of collagen it was conceivable that the process was time dependent rather than following the physiological age. However, comparison of the rates of decrease in the reducible cross-links showed the process to be virtually complete at maturity; 4–5 years for the bovine and 17–20 years for the human tissues. Using tendon as an example, fig. 2 shows the rates of disappearance of hydroxylysinonorleucine and syndesine.

These results clearly demonstrate that the aldehyde derived cross-links do indeed change with age and that these reducible bonds observed in young tissues are probably intermediate cross-links as originally proposed [6]. Since the changes are virtually complete at maturity they must be an essential part of the growth process rather than an ageing phenomena. Similar changes occurring during the maturation of rat, bovine and human tissues confirm that the cross-linking process follows the physiological rather than the temporal age of the animal.

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