DISEASES AFFECTING PRIMARILY COLLAGEN FIBRES

Thesis

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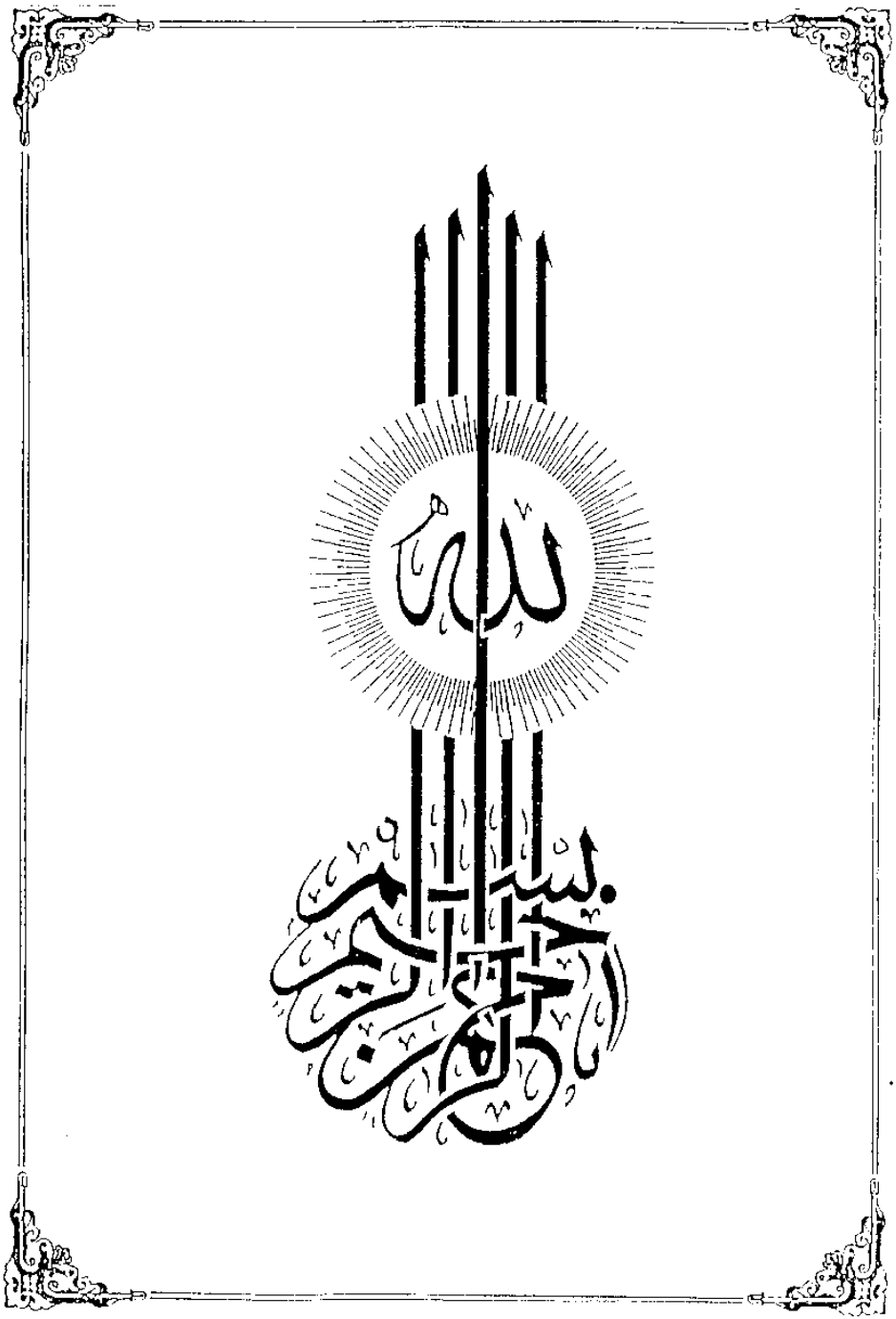

A C K N O W L E D G M E N T

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C O N T E N T S

INTRODUCTION

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Collagen constitutes approximately one third of the body's total protein, and changes in synthesis and/or degradation of collagen occur in nearly every disease process. There are also a number of specific diseases of collagen in which the collagen fibres are primarily affected. Fortunately, during the last decade much progress has occurred in understanding of the metabolism of collagen. Much of this progress is discussed in a number of recent books and reviews. A significant part of this progress has been due to studies of the specific defects in the metabolism of collagen in disease of both man and domestic animals. Therefore, this review will briefly discuss the current state of knowledge of structure, biosynthesis, and degradation of collagen. This discussion will serve as a basis for a review of the primary diseases of collagen. The Ehlers-Danlos syndromes are considered as the primary examples of this group of diseases, therefore, they will be discussed in detail.

COLLAGEN FIBRES

Structure of collagen

Collagen is the major fibrillar component of most connective tissues. It comprises about 70% of the dry weight of human skin and about one third of the total human protein (Uitto et al., 1981).

Structure of collagen molecule

As a result of combined physico-chemical and electron microscopic studies, it has been concluded that collagen is formed of fundamental units or molecules called tropocollagen. This basic collagen molecule has an approximate molecular weight of about 290,000, and it is composed of three polypeptide chains, each having a molecular weight of about 94,000. These three polypeptides are called α-chains. Each of these chains is coiled into a left-handed helix with about three amino acids per turn. The three helical chains are twisted around each other into a right-handed super-helix to form a rigid structure similar to the strands of a rope. This unusual triple-helical conformation gives the molecule a rigid rod-like shape with about 280-300 nm. long and 1.5 nm. in diameter (Uitto and Lichtenstein, 1976; Ebling, 1979; Prockop et al., 1979).

Each α-chain of collagen has approximately 1,000 amino-acids. Glycine, the smallest amino acid, accounts for about one third of the total number of amino acids.

Sequence studies of the amino acids in α-chains have demonstrated that glycine is evenly spaced in the polypeptides occuping every third position. The α-chains of collagen can therefore be considered as a repeating triplet represented by the formula $(Gly\text{-}X\text{-}Y)_{333}$. The X and Y positions can be occupied by a variety of amino acids except glycine, but frequently proline is found in the X position while hydroxyproline is often present in the Y position. These two imino acids account for about 20-25% of the total amino composition of collagen (Uitto and Lichtenstein, 1976; Prockop et al., 1979).

Structure of collagen fibres

The morphological appearance of collagen fibres of different tissues, when examined by histological staining techniques or electron microscopy, is variable. This variability is probably explained by the fact that the tissue distribution of different types of collagen varies (Uitto et al., 1981). The difference in morphological appearance will be mentioned later with collagen types.

Under the ordinary light-microscope, in unstained preperations of loose connective tissue, collagen fibres appear as colourless, branching wavy bundles about 1-15µm in thickness and of indefinite length. The bundles run in all directions and their endings merge with other components of connective tissue and cannot be seen. Collagen fibres appear white in dense fibrous tissue,

therefore, they are sometimes called white fibres. There is no specific staining reaction for collagen fibres. They are stained pink with eosin, blue with Mallory's stain, red with van Geison' stain, and brown with silver staining methods. At high magnification, collagen fibres appear longitudinally striated and composed of fibrils with a diameter of 0.2 - 0.5 μm. The diameter of the fibres dependes on the number of fibrils they contain (Bloom and Fawcett, 1975; Junqueira and Carneiro, 1980).

Electron Microscopy of collagen fibrils

Under the electron microscope collagen fibrils appear to consist of smaller strands which may represent "microfibrils". These microfibrils are only about 7 nm. in diameter, which could be accounted for by 4-8 collagen molecules in cross section. There is still some controversy, however, about whether the fibrils are composed of discrete microfibrils or whether the cross-sectional lattice of the fibril consists of random packing of individual molecules (Prockop et al., 1979).

Electron microscope discloses that collagen fibrils exhibit what is termed axial periodicity, which means they reveal cross-striations or bands units repeating every 64 nm along their lengths. This periodicity may vary from 60-70 nm in different preparations. The most obvious feature of the cross-banding is the sequence of dark and light bands

i.e. a dark cross band followed by a light cross band and so on. The distance between cross-striations is usually referred to as "D space" which is composed of one dark band plus one light band. The dark band is 0.6 D and the light band is 0.4 D. The collagen molecule is 4.4 D, therefore it extends across five light bands and four dark bands. The cross bands are seen most clearly in negatively stained preparations of collagen fibrils (Ham and Cormack, 1979; Prockop et al., 1979).

The axial periodicity could theoretically be attained if collagen molecules are arranged side by side in a paralled but staggered fashion, with each molecule overlaping the neighboring one by one quarter of its length but without meeting the ends of the molecules ahead of or behind it (Fig. 1). There is a 0.6 D space or hole between each succeeding linearly associated molecule. The electron dense stains enters the places where collagen fibrils are least dense in content. The dark bands are not dense in content as the light bands, therefore, they retain more of the stain used in electron microscopic studies. Each light band, as can be seen from the diagram (Fig. 1), has five tropocollagen molecules passing through it for every four that pass through a dark band (Ham and Cormack, 1979).

If a solution of collagen and α -acid glycoprotein of serum is dialyzed against water, the collagen fibres

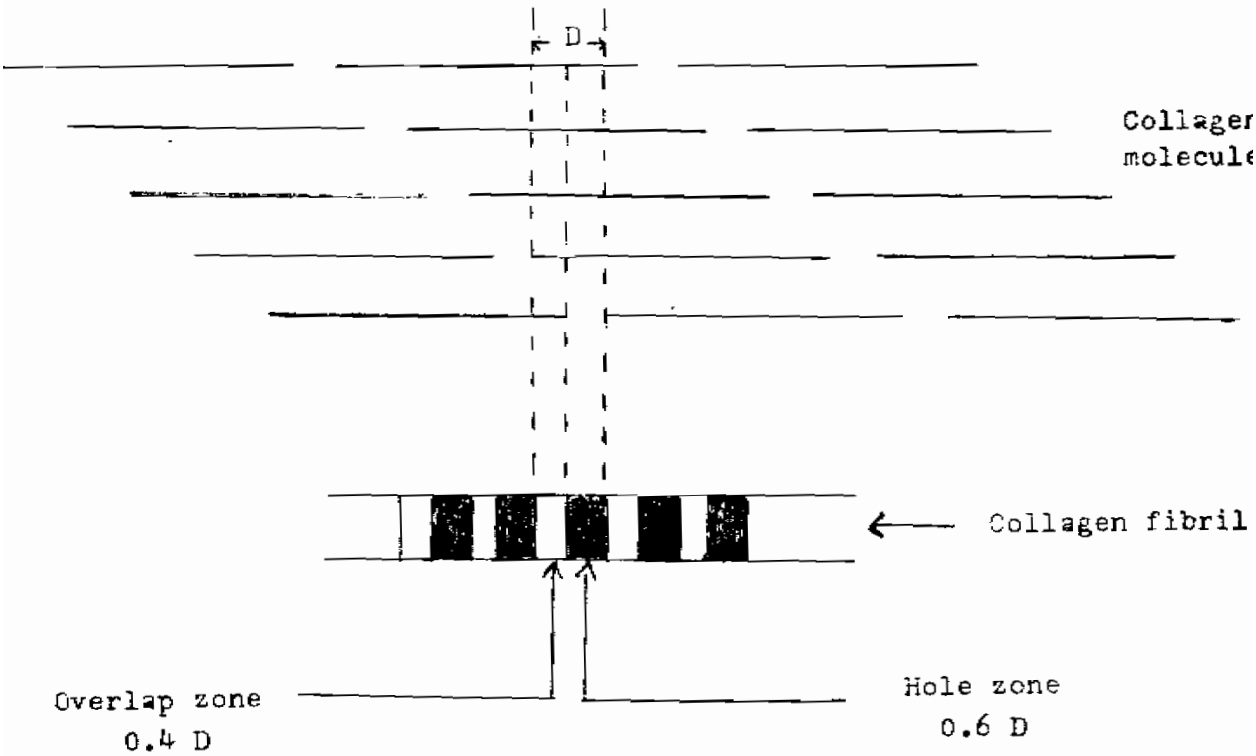

Figure 1. Schematic Representation of the Structure of a Collagen Fibril. After Prockop et al. (1979).

that are formed have a period 240 nm instead of the 64 nm of the native fibres. This form is called fibrous long spacing collagen. Precipitation from acid solution by addition of adenosine triphosphate (ATP) yields segments about 240 nm long instead of fibres. This form is called segment long spacing collagen. In both of the long spacing forms, the collagen molecules are believed to come together side to side, in register, so that the length of the period, or of the segment, is approximately the same as the length of collagen molecules (Bloom and Fawcett, 1975).

Types of collagen

The term collagen has recently been expanded to include at least seven and possibly ten genetically different types of collagen molecules. These different molecules may contain either one type of α-chain or more of genetically different α-chains (Table I) (Burgeson, 1982).

These collagen types actually consist a family of proteins which are structurally related, but there is considerable variation in the content and sequence of amino acids in each one of them (Table II). This variation occurs in the amino acids which occupy the X and Y positions of the repeating Gly-X-Y triplets (Prockop et al., 1979; Bornstein and Sage, 1980). The differences between collagen types clearly indicate that there are several different genes coding for α-chains with variable amino acid sequence (Miller, 1976).