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THE STABILITY OF EXTRACTED COLLAGEN MOLECULES FROM HUMAN PERIODONTAL MEMBRANE

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INTRODUCTION

Former investigations concerning contraction temperature and the solubility of collagen in human periodontal membrane indicated deficiencies in the organization of the collagen molecules in those materials classified as diseased (*Paunio*, 1969). The results suggested that intermolecular and/or intramolecular cross-links in the collagen of diseased periodontal membrane were less developed than those of the control material.

The aim of the present study was to investigate the stability of the collagen molecules in periodontal membrane with special reference to solubility, age and periodontal condition.

MATERIAL AND METHODS

The material was collected from extracted human teeth as earlier described (*Paunio*, 1969). The samples were pooled according to the age of the patients and to the periodontal condition, equally from females and males. The location of the epithelial attachment was employed as a clinical criterion when the samples were classified (*Paunio*, 1969). If the epithelium of the gingiva was not attached to the cementoenamel junction, the material was classified as diseased. The teeth with normal epithelial attachment were used as control material.

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Unless otherwise stated the reagents were obtained from E. Merck AG, Darmstadt, W.-Germany.

Every pool (about 150 mg) was divided into 2 parts and homogenized in 0.5 M acetic acid (50 ml) (30 sec, at appr. +2°C). The homogenate of the first part of the pools was shaken for 4hr at +4°C, 130 rev/min (Bühler shaker, Type Sm2; Tübingen, W.-Germany). The homogenate of the second part of the pools was shaken 24 hr (+4°C). The homogenate was then centrifuged (27000 × g, +2°C). The collagen in the supernatant was precipitated and purified with repeated salt precipitations (*Heikkinen,* 1968). Sodium chloride was added to the supernatant to give a 15 % (w/v) solution. The mixtures were centrifuged and the precipitate dissolved in 0.5 M acetic acid. The collagen was reprecipitated with sodium chloride to give a 10 % (w/v) solution. In order to avoid a local increase of the ionic strentgh sodium chloride was carefully added to the solution, and the procedure was repeated. The solid residue was then dissolved in 0.1 M acetic acid (15 ml) and dialyzed against distilled water (72 hr, +4°C). The precipitate was dissolved in sodium acetate buffer (pH 4.8, $\mu = 0.017$).

The fractionation of the purified collagen was carried out according to *Näntö et al.* (1965) by starch-gel electrophoresis (Starch-hydrolysed, Connaught Medical Research Lab., Toronto, Canada). The samples (50 μl, 0.2 %) were denatured at +40°C for 15 min just prior to electrophoresis. The solution of denatured collagen was imbibed into filter paper which was then placed in a slot of the gel. The plastic trough and the electrode vessels were constructed at the workshop of the institute. The gels were stained for 20 min at +85°C in a solution of 50 % (v/v) aqueous glycerol containing 0.1 % water soluble nigrosine (Fluka AG, Buchs, Switzerland) and stored in this solution. The gels were scanned with a double beam densitometer (Joyce, Loebl, Co., Ltd., Gateshead, England) in the ratio 3/1 and by using a special filter for nigrosine.

Collagen hydrolysates were prepared and analyzed on a Beckman Unichrom Amino Acid Analyzer according to instructions (Beckman Instruction Manual for Beckman Unichrom Amino Acid Analyzer, 1966). The hydroxyproline determinations were carried out according to *Stegeman* and *Woessner* as described by *Pikkarainen* (1968).

RESULTS

The electropherogram in Fig. 1, the densitograms in Fig. 2 and the amino acid analyses in Table I present the results.

The α_2-chain in the collagen molecule moved most rapidly towards the catode. The α_2-bands in the pherograms of the samples dissolved 4 hr in 0.5 M

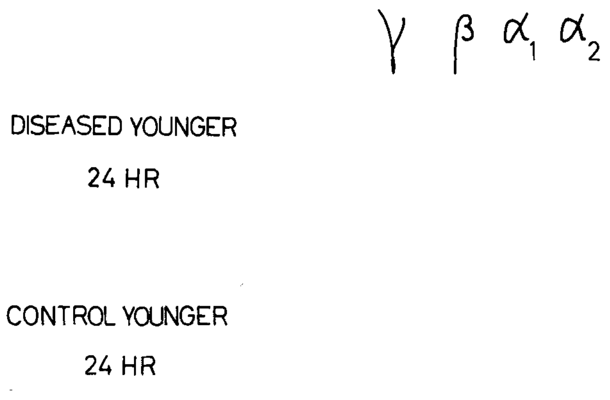


Fig. 1. Electropherograms of the collagen components extracted from the periodontal membrane of man with 0.5 M acetic acid.
Conditions in electrophoresis: sodium acetate buffer, pH 4.8, $\mu = 0.017$, running time 5 hr., voltage gradient 9 V/cm, the electrophoresis was carried out at +37°C.

acetic acid had a high dye intensity in relation to the other fractions (α_1, β_{11}, $\beta_{12}$and γ). In the 24 hr samples the relative share of α_2-band decreased, giving way to larger components (β- and γ-fractions) (Figs. 1 and 2). This refers especially to samples obtained from diseased patients.

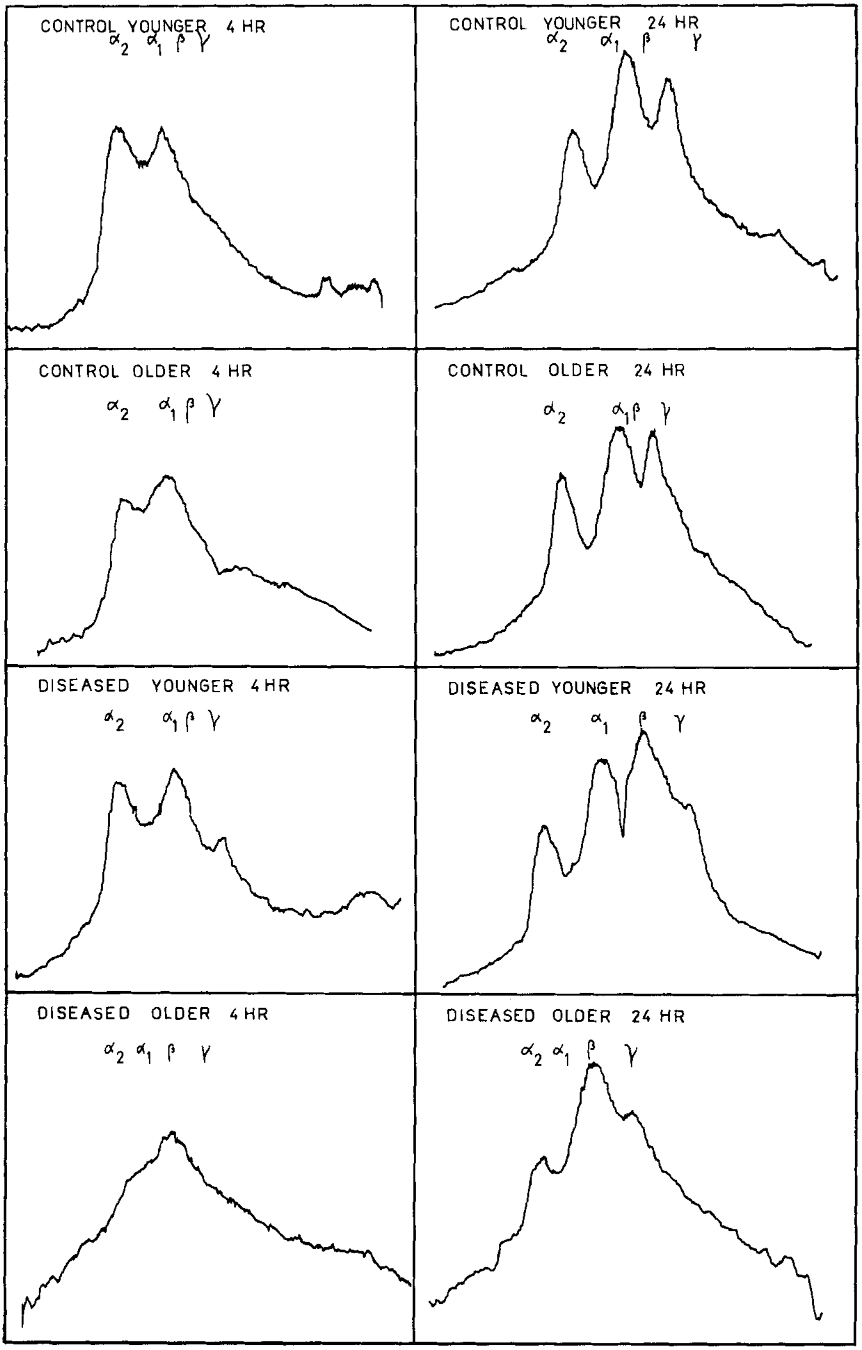
The densitograms of samples from older patients and classified as diseased indicate a clear difference when compared to the pherograms of samples obtained from young, diseased patients (Fig. 2). The α_2-pattern was weak

Table I

The content of hydroxyproline (Hypro), proline, (Pro) and glycine (Gly) in the collagen samples extracted from the periodontal membrane.

			Hypro μM/ml	Pro μM/ml	Gly μM/ml	4 hr-samples Hypro/Gly*	24 hr-samples Hypro/Gly**
Control	younger	4 hr	0.707	0.647	1.333	0.53	
»	»	24 hr	0.686	0.490	1.056		0.65
	older	4 hr	0.540	0.776	1.228	0.45	
»	»	24 hr	0.265	0.218	0.412		0.64
Diseased	younger	4 hr	0.560	0.584	1.052	0.53	
»	»	24 hr	0.549	0.424	0.920		0.60
	older	4 hr	0.793	0.992	1.700	0.47	
»	»	24 hr	0.828	0.584	1.270		0.65

Mean *0.50 (S.D. = 0.02), **0.64 (S.D. = 0.04)


CONTROL YOUNGER 4 HR
α2 α1 β γ
CONTROL YOUNGER 24 HR
α2 α1 β γ
CONTROL OLDER 4 HR
α2 α1 β γ
CONTROL OLDER 24 HR
α2 α1 β γ
DISEASED YOUNGER 4 HR
α2 α1 β γ
DISEASED YOUNGER 24 HR
α2 α1 β γ
DISEASED OLDER 4 HR
α2 α1 β γ
DISEASED OLDER 24 HR
α2 α1 β γ

compared to the other patterns. In the control material this phenomenon was not detectable to the same extent (Fig. 2).

The amino acid analyses of both diseased and control samples showed no noticeable differences in the ratio of glycine, proline and hydroxyproline (Table I). On the other hand the ratio hydroxyproline/glycine was lower in the 4 hr-samples than in the 24 hr-samples.

DISCUSSION

Collagen fibrils are composed of specific aggregates of collagen molecules with a molecular weight about 300 000. The molecule is three stranded, each strand being in a modified polyproline helix. The three strands (α_1, α_2 and α_3) are wound together as a triple helix. The structure is stabilized by a regular array of interchain hydrogen bonds and cross-links, together with stereochemical restrictions which derive from the high content of pyrrolidine rings found in collagen (proline and hydroxyproline) (*Piez,* 1966). When a solution of native collagen is denatured by heating, the chains of the molecule uncoil and disassociate.

Solutions of denatured acid-soluble collagen contains in addition to the α- and β- (two aggregated α-chains) components a small proportion of a third and larger component designated γ (*Schleyer,* 1962). The results of the present study suggest that by consecutive extraction of the periodontal membrane collagen, an increase of larger aggregates can be observed. The trend was the same as observed by *Heikkinen and Kulonen* (1966) in rat skin. The larger aggregates were hardly detectable in the pherograms of 4 hr-samples and they resemble the pherograms of alkali- and salt-soluble collagen of rat (*Heikkinen,* 1969). The results of solubility experiments depend on conditions and methods that are difficult to standardize. Therefore such terms as alkali-soluble collagen, neutral salt-soluble collagen and acid-soluble collagen do not precisely define a given collagen fraction.

The results of the present investigation indicate that samples from older patients, especially diseased, contain a greater amount of larger aggregates than samples from younger patients. The earlier results (*Paunio,* 1969) indicated an increased solubility of the collagen in diseased periodontal membrane. This might explain the amount of larger aggregates detected in the pherograms. During the given time intervals (4 hr and 24 hr) more collagen in larger aggregates was probably solubilized. The results confirm

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Fig. 2. Desitograms of the electropherograms of the collagen components extracted from the periodontal membrane of man.

The material consisted of 4 pools divided into 2 parts from 85 individuals comprising age ranges of $\leqq$ 35 (younger) and $\leqq$ (older) years respectively.

the observations made by *Heikkinen* and *Kulonen* (1964) that the stability of collagen molecules increases with age. They observed an increase of the β- and γ-components in the soluble collagen of rat tail tendonand rat skin from electrophoretic patterns. On the other hand, when skinsamples from men of different ages were extracted, no differences in the proportions of α- and β-components were found (*Bakerman,* 1964).

The results of the amino acid analyses in the present study are similar to those in animal experiments concerning the amino acid composition in neutral salt-soluble and acid-soluble collagen (cf. *Eastoe,* 1967). *Eastoe* suggested that conditions for the extraction of a neutral salt-soluble fraction favour the collagen being more contaminated with protein impurities than for an acid-soluble fraction. This probably explains the relatively deficiency of hydroxyproline content in 4 hr-samples in the present study as compared to the 24 hr-samples.

The results in the present study indicate that increased solubility and decreased thermal stability of collagen in the diseased human periodontal membrane samples (*Paunio,* 1969) depend on deficiencies in the intermolecular cross-links rather than in the intramolecular cross-links.

SUMMARY

The aim of the present study was to investigate the stability of the collagen molecules in the periodontal membrane with special reference to solubility, age, and periodontal condition.

The material was collected from extracted human teeth. The samples were pooled according to the age of the patients and to the periodontal condition, equally from females and males. The collagen soluble in 0.5 M acetic acid (4 hr and 24 hr) was purified with repeating salt precipitations. The fractionation of the purified collagen was carried out by starch-gel electrophoresis. The amino acids were determined by an amino acid analyzer.

The elextropherograms show that in the 24 hr-samples the relative share of α_2-band decreased giving place to bigger components (β- and γ-fractions). This concerns especially the samples obtained from diseased patients. The ratio hydroxyproline/glycine was lower in the 4 hr-samples than in 24 hr-samples.

The results in the present study indicate that the increasing solubility and decreasing thermal stability of the collagen in diseased periodontal membrane samples depend on the deficiencies in intermolecular cross-links rather than on intramolecular cross-links.

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RÉSUMÉ

STABILITÉ DES MOLÉCULES DU COLLAGÈNE EXTRAIT DU DESMODONTE HUMAIN

Le présent travail a eu pour but l'étude de la stabilité des molécules de collagène dans le desmodonte, plus particulièrement par rapport à la solubilité, à l'âge et à l'état du desmodonte.

Le matériel a été recueilli à partir de dents humaines extraites. Les échantillons ont été réunis dans des pools correspondant à l'âge des patients et à l'état du desmodonte, en quantités égales à partir des deux sexes. Le collagène soluble dans l'acide acétique 0,5 M (4 heures et 24 heures) a été purifié par des précipitations de sels répétées. Le fractionnement du collagène purifié a été effectué par électrophorèse sur gel d'amidon. Les aminoacides ont été déterminés par un analyseur d'aminoacides.

Les résultats de l'électrophorèse ont montré que la part relative de la bande α_2 dans les échantillons de 24 heures diminuait, laissant la place à des éléments plus grands (fractions β et γ). Ce fait concerne particulièrement les échantillons provenant de patients présentant une affection. Le rapport hydroxyproline/glycine était moins élevé dans les échantillons de 4 heures que dans ceux de 24 heures.

Les résultats de la présente étude indiquent que l'augmentation de la solubilité et la diminution de la stabilité thermique du collagène tiennent à des défauts dans les liaisons croisées intermoléculaires plutôt qu'au niveau des liaisons croisées intramoléculaires.

ZUSAMMENFASSUNG

DIE STABILITÄT VON AUS MENSCHLICHER WURZELHAUT EXTRAHIERTEN KOLLAGENMOLEKULEN

Es war das Ziel der vorliegenden Untersuchung, die Stabilität der Kollagenmoleküle im Desmodont unter besonderer Berücksichtigung der Löslichkeit, Alters und der periodontalen Verhältnisse zu erforschen.

Das Material wurde aus extrahierten menschlichen Zähnen gewonnen. Die Proben wurden nach Alter der Patienten und nach periodontalen Verhälnissen, sowie nach Geschlecht getrennt geordnet. Das Kollagen, gelöst in 0,5 M Essigsäure (4 und 24 Stunden), wurde mittels wiederholter Aussalzung purifiziert. Die Trennung des purifizierten Kollagens wurde durch Stärkegel-Elektrophorese durchgeführt. Die Aminosäuren wurden durch einen Aminosäuren-Analysator bestimmt.

Die Elektropherogramme weisen auf, dass in den 24-Stunden-Proben der relative Anteil des α_2-Bands zugunsten grösserer Komponenten (β- und γ -Fraktion) abnahm. Dies betrifft besonders die Proben, die von erkrankten Patienten erhalten wurden. Das Verhältnis Hydroxyprolin/ Glycin war in den 4-Stunden-Proben niedriger als in den 24-Stunden-Proben.

Die Ergebnisse der vorliegenden Untersuchung zeigen, dass die steigende Löslichkeit und abnehmende Hitzebeständigkeit des Kollagens bei Proben erkrankten Desmodonts eher durch fehlende intermolekulare Verknüpfung als durch intramolekulare Verknüpfung hervorgerufen werden.

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