Biochemical Characterization of Collagens Synthesized by Fibroblasts Derived from Normal and Diseased Human Gingiva

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Fibroblasts obtained from healthy and diseased human gingiva were labeled with radioactive amino acids and the collagenous proteins synthesized were studied. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of untreated, reduced, and pepsin-treated proteins of the medium and cell extract showed that the collagenous proteins synthesized by these cells exist in the precursor form. Type I collagen was the chief constituent. In addition, cells from normal tissue synthesized type III collagen in amounts varying from 5 to 30%. Type III collagen was not detected in the cultures of fibroblasts from diseased gingiva, however, an additional collagen fractionated between 2.5 to 5.0 m NaCl and accounted for 22 to 29% of the total. This collagen had an α1/α2 ratio of 8.6 and hydroxylysine/lysine ratio and cyanogen bromide peptide pattern were similar to that of α1[Ⅰ]. It is concluded that the fibroblasts derived from diseased gingiva synthesize a collagen of composition (α1)3, probably of type I.

Collagen is an essential component of all connective tissues. It is composed of three α chains of 1,050 amino acids each, bound into a helix of polypeptide II type (1). Collagen is synthesized in the form of a precursor known as procollagen which is converted extracellularly to collagen by limited proteolysis (2). At least four different collagen types have been described. Type I collagen consists of two α1 chains and one α2 chain and is present in all connective tissues except cartilage (3). Type II, III, and IV molecules are composed of three α1 chains and these are present in cartilage, fetal tissues, and basement lamina, respectively (4-6).

Collagen is the principal component of human gingiva, and its most important structural component (7). The collagen fibers of this tissue exhibit an exquisite degree and pattern of organization (8) and serve the unique function of maintaining the organic union between the soft tissue and the calcified tooth surface. The gingival collagen seems to be unusual in that it turns over at a very high rate even in the adult, at least in subhuman primates (9). At a very early stage of inflammatory gingival and periodontal disease, the architecture of the gingival collagen fiber bundles is disrupted and almost 70% of the total collagen is lost (10, 11). As the disease progresses, this loss persists in some areas, while in the adjacent areas fibrosis occurs. These alterations are a cardinal feature of periodontal disease as well as of numerous other chronic inflammatory lesions.

The biochemical characteristics of collagen making up the normal marginal gingiva have not been defined and the nature of the collagenous alteration accompanying inflammatory disease remains poorly understood. We have analyzed the biochemical nature of the collagenous material synthesized in vitro by fibroblasts obtained from normal human gingiva and cells from the gingiva of an individual with severe chronic periodontitis.

**Experimental Procedure**

**Materials**

Type I collagen of rat and human fetal skin was prepared by the method of Piers et al. (12) and Type II collagen from articular cartilage of aborted human fetuses by the method of Miller (13). Human type III collagen was a gift of Dr. E. J. Miller; additional material was prepared from human fetal skin according to the method of Chuang and Miller (14).

All radioactive amino acids and Aquasol were obtained from New England Nuclear, Boston, Mass. Pepsin (specific activity 3250 units/mg) was the product of Worthington Biochemical Corp., Freehold, N.J. β-Aminopropionitrile, iodoacetic acid, and mercaptoethanol were obtained from Calbiochem, La Jolla, Ca., and ion exchange celluloses from Whatman Biochemicals Ltd., Maidstone, Kent, United Kingdom. Chemicals for electrophoresis were the products of Bio-Rad, Richmond, Ca. All other chemicals not listed above were of analytical grade and purchased from Mallinckrodt, Baker, or Fisher Chemical.

**Methods**

**Cell Culture—**Fibroblasts were obtained from biopsies of the interproximal gingival papilla between the maxillary premolars of an individual with clinically and radiographically normal periodontal tissues who had undergone dental prophylaxis and 3 weeks of intensive oral hygiene, and from a comparable site in an individual with severe chronic periodontitis with extensive bone loss. Using standard techniques (15), fibroblasts were grown and maintained in 10-cm² plastic culture flasks in Dulbecco-Vogt medium. Only cells between 5 and 15 doublings were used in the experiments described. Confluent monolayer cultures were labeled 10 days after passage with 5 μCi/ml of

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in Dulbecco-Vogt medium lacking these amino acids and serum but containing 50 \( \mu \)g/ml each of ascorbic acid and beta-aminopropionitrile. The latter compound specifically inhibits lysyl oxidase and prevents the newly synthesized collagen from becoming cross-linked (16). Cultures were incubated for 24 h at 37°C in an atmosphere of 5% CO2 and 96% air. The medium was harvested and the cell layer extracted twice by stirring overnight in 5 ml flask of 0.5 M acetic acid at 4°C. The insoluble residue was removed by centrifugation and the two extracts combined. Unincorporated radioactivity was eliminated by dialysis, and the medium, cell extract, and insoluble residue were analyzed separately.

Limited Pepsin Digestion—The cell extract in 0.5 M acetic acid or medium and other samples previously dialyzed against 0.5 M acetic acid, were treated with 100 \( \mu \)g/ml of pepsin for 6 h at 15°C (15). The pH was then adjusted to 7.2 to 8.0 with NaOH to inactivate the pepsin.

CM-cellulose Chromatography—Separation of the various collagen \( \alpha \) chains was done on a CM-cellulose column (0.9 x 9.0 cm) at 42°C as described by Piers et al. (12) using 0.02 M sodium acetate buffer, pH 4.5, containing 1 M urea with a NaCl gradient of 0 to 110 mM in a total volume of 200 ml of buffer. The column was standardized with human type I, II, and III collagens. During the chromatography, the samples contained 5 mg of lathyritic rat skin collagen as internal marker and carrier. Cyanogen bromide peptides were separated on column (0.9 x 13 cm) at 42°C with NaCl gradient of 0.02 to 0.17 M in 200 ml of 0.02 M sodium acetate buffer, pH 3.8 (17).

Reduction and Alkylation—Samples were subjected to pepsin digestion at room temperature in 0.05 M Tris-HCl buffer, pH 7.4, containing 8 M urea and 50 mM mercaptoethanol (18). After 6 h they were cooled in ice and stirred for 30 min with 100 M isoacetic acid. The samples were then dialyzed to remove urea and lyophilized.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis—a and pro-\( \alpha \)-chains of collagen were separated in 5% gels by the method of Weber and Osborn (19). The electrophoresis was done in the absence of mercaptoethanol except when specified. Each gel contained 50 \( \mu \)g of lathyritic rat skin collagen as internal marker and carrier. Molecular weights were calculated from the migration of collagen chains. Resolution of cyanogen bromide peptides was achieved in 7.5% gels by excess (w/w) of cyanogen bromide was added and the collagen digested contained 5 to 10 mg of purified human al [I] as carrier. A 5-fold excess (w/w) of cyanogen bromide was added and the collagen digested for 4 h at 30°C (5). Human type II collagen was also digested similarly.

Amino Acid Analysis—Samples were hydrolyzed for 24 h in constant boiling HCl at 105°C and the radioactive amino acids separated on a Beckman 120C amino acid analyzer equipped with a stream-splitting device. Fractions were collected and the radioactivity in fractions measured by counting aliquots in Aquasol.

RESULTS

Characterization of Collagens Synthesized by Fibroblasts from Normal Gingiva—Incorporation of the radioactive

\[ ^{3}H \text{proline or} \quad [^{3}H] \text{glycine or both, or} \quad 2 \mu \text{Ci/ml of} \quad [U \text{ } ^{14} \text{C}] \text{proline} \]

in Dulbecco-Vogt medium lacking these amino acids and serum but containing 50 \( \mu \)g/ml each of ascorbic acid and beta-aminopropionitrile. The latter compound specifically inhibits lysyl oxidase and prevents the newly synthesized collagen from becoming cross-linked (16). Cultures were incubated for 24 h at 37°C in an atmosphere of 5% CO2 and 96% air. The medium was harvested and the cell layer extracted twice by stirring overnight in 5 ml flask of 0.5 M acetic acid at 4°C. The insoluble residue was removed by centrifugation and the two extracts combined. Unincorporated radioactivity was eliminated by dialysis, and the medium, cell extract, and insoluble residue were analyzed separately.

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Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of medium proteins and 0.5 M acetic acid extract of cultures of normal human gingival fibroblasts labeled with [14C]proline. Equal aliquots of medium or cell extract were treated and subjected to electrophoresis. The left panel (A, B, C) represents the data for medium and the right panel (D, E, F) represents cell extract. A and D, untreated; B and E, after reduction and alkylation; C and F, after limited digestion with pepsin. The mobilities of γ, β, and α chains of rat skin collagen, which were used as internal markers, are indicated. Radioactivity of each 1-mm slice is shown.

Fig. 2. CM-cellulose chromatography of medium collagen produced by fibroblasts from normal gingiva and labeled with [14C]proline and [2-14C]glycine. A, untreated; 297.1 x 10^3 cpm were loaded and 5.6 x 10^4 cpm were recovered in the fractions. B, reduced and alkylated; 74.6 x 10^3 cpm were loaded. C, digested with pepsin; 158 x 10^3 cpm were loaded. Fractions of 6.9 ml were collected and the radioactivity of 1.0 ml portions is shown. In C the radioactivity of α(I) [I], α(III) [III], and α2 peaks were 3080, 160, and 1350 cpm, respectively (α1/α2 = 2.3). The top figure represents the ultraviolet scan of rat skin collagen; human α2 elutes slightly later and the elution of human α1, α1 [III], and α2 chains are indicated.

Table II

<table>
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<tr>
<th>Fraction</th>
<th>Radioactivity, total proteins</th>
<th>Radioactivity, collagen</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>cpm x 10^3</td>
<td>Per cent of total</td>
</tr>
<tr>
<td>1.5 M NaCl pellet</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>1.8-2.5 M NaCl</td>
<td>9</td>
<td>41</td>
</tr>
<tr>
<td>2.5-5.0 M NaCl</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

The labeled culture medium collagen was subjected to limited pepsin digestion and fractionated with NaCl, as described under "Experimental Procedures." The fractions were desalted by dialysis and subjected to CM-cellulose chromatography. The total collagen was calculated by adding the radioactivity under α1, α1 [III], and α2 peaks.

*Total radioactivity under α1 [I] plus α2 peaks.

The material synthesized by the gingival cells was eluted from a DEAE-cellulose column using buffer containing 2 M urea under conditions expected to separate Type I and III procollagens (18). However, only 2 to 3% of the material loaded was recovered. The addition of 6 M urea to the buffer system permitted recovery of about 40% of the material, but the separation of procollagens was poor. Therefore, the material was subjected to pepsin digestion and salt fractionation by the methods of Trelstad et al. (21) and Chung and Miller (14), followed by characterization using CM-cellulose chromatography. In a typical experiment, the 5.0 M NaCl supernatant contained 47% of the total radioactivity but only negligible amounts of collagenous protein (Table II, Fig. 3A). The 2.5 to 5.0 M NaCl fraction, which is expected to contain type II molecules if present, contained only 4% of the total collagenous protein and on CM-cellulose chromatography revealed an α1/α2 chain ratio of 2.8 (Table II, Fig. 3B); thus this fraction appeared to be type I collagen. The 1.5 to 2.5 M NaCl fraction, which is expected to contain type I molecules, had 87% of the total collagen, eluted from CM-cellulose as α1 and α2 chains with a ratio of 2.6 (Fig. 3C), indicating that it is type I collagen. Approximately 8% of the total collagenous material appeared in the 1.5 M NaCl fraction, which is expected to contain type III collagen. On CM-cellulose chromatography, this fraction revealed a peak eluting in the region of type III chains and in the experiment presented this peak accounted for 5% of the total collagen (Table II, Fig. 3D). To confirm the identity of the putative type III chains, the fractions under this peak were pooled, desalted, and examined electrophoretically with and without reduction and alkylation. Since type III molecules

*The term "α1" has been used in reference to the α1 chains that will elute on CM-cellulose chromatography in the position of rat skin α1 [I]. This fraction might derive from type I (14), II (4), or IV (6) collagens, and therefore may be a mixture of more than one type. However, the presence of α2 and α1/α2 ratio of 2.0 would indicate that it is derived from type I collagen. When necessary, we have indicated the type of collagen in parentheses.
Collagens Synthesized by Gingiva Fibroblasts in Vitro

Radioactive protein were subjected to electrophoresis, either untreated (O—O) or after reduction and alkylation (■■■). MSH, mercaptoethanol.

Collagen of Cell Residue of Fibroblasts from Normal Human Gingiva—As noted in Table I, about 5% of the total collagenous protein synthesized by the normal gingival fibroblasts remained in the cell residue after extraction with 0.5 M acetic acid. The residue was suspended in 0.5 M acetic acid and solubilized by overnight digestion at 4°C with pepsin (1 mg/ml) (6, 13). The solubilized material was subjected to salt precipitation and analysis by CM-cellulose chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Only α1(I) and α2 chains, in a ratio of 2.0 to 2.5, were found, thereby indicating that only type I collagen is present in the cell residue. The small amount of radioactive collagen obtained precluded further characterization by cyanogen bromide digestion.

Characterization of Collagens Synthesized by Fibroblasts Derived from Diseased Gingiva—Morphologically, no differences were noted between the cells obtained from normal gingiva and those from the diseased tissue. Although cell counts were not done, the cells from the diseased tissue appeared to grow more slowly than those from the normal tissue and, under identical transfer conditions, more time was required for the former to become confluent.³ Cultures of the two types did not differ significantly in their capacity to synthesize protein or collagen (Table I), although more of the collagenous material synthesized by the cells from the abnormal tissue was present in the medium (Table I). Distribution of collagenous protein in the absence of β-aminopropionitrile was not evaluated.

Radioactive proteins synthesized by the cells from diseased gingiva remained near the origin when examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 5A). After reduction and alkylation, peaks of lower molecular weight appeared and these migrated in the region of pro-α1 (M_r = 145,000) and pro-α2 (M_r = 118,000) chains (Fig. 5B).³ Material subject to limited pepsin digestion migrated in the region of α1 and α2 chains (Fig. 5C). The radioactive proteins of the cell extract behaved in the same manner (not shown). Thus, as in the case of normal cells, procollagen rather than collagen predominated in the cultures.

Attempts at elution of the untreated medium collagen from CM-cellulose columns were not successful; very little material was recovered under the α chain peaks (Fig. 6A). Recoveries were not improved by reduction and alkylation (Fig. 6B), but following limited pepsin digestion discrete peaks of α1 and α2 chains were present (Fig. 6C). The α1/α2 ratio ranged from 3.4 to 4.8, a value considerably higher than 2.0 expected for type I collagen, and indicating possible molecular heterogeneity. Therefore, the pepsin-digested material was subjected to salt fractionation and characterization (Table III, Fig. 7). The 1.5 M

³ When cells from one confluent flask were transferred to two flasks, the normal cells became confluent in 3 to 4 days, the abnormal cells in 5 to 7 days.

⁴ Reduction and alkylation yields considerably more pro-α2 than expected of type I collagen. This could be due to the presence of partially degraded pro-α1-chain along with pro-α2. The presence of low molecular weight peaks (see 4- to 5-cm portion of the gel in Fig. 5B) supports this possibility. Alternatively, the precursor of the unusual α1 chain described later may migrate along with pro-α2.
FIG. 5. Sodium dodecyl sulfate polyacrylamide gel electrophoretic pattern of medium from cells of diseased human gingiva labeled with \(^{[3H]}\)proline. Equal portions of medium were treated and applied to gels. A, untreated; B, reduced and alkylated; C, digested with pepsin. Radioactivity of each 2-mm slice is shown, thus resolution is not as good as in Fig. 1. The migration of collagen chains is indicated.

FIG. 6. CM-cellulose chromatography of medium collagen produced by cells of the diseased gingiva and labeled with \(^{[3H]}\)proline. Fractions of 6.9 ml were collected and 1.0-ml portions counted. The top figure shows the ultraviolet scan of rat skin collagen carrier. The elution of human \(\alpha_1\), \(\alpha_1[III]\), and \(\alpha_2\) is shown. In this system \(\alpha_1\), \(\alpha_1[II]\), and \(\alpha_1[IV]\) chains elute in the same position. A, untreated; 94.5 \times 10^5 cpm were loaded. B, reduced and alkylated; 73.6 \times 10^5 cpm were separated. C, after limited digestion with pepsin; 58.7 \times 10^5 cpm were loaded. The radioactivity under \(\alpha_1\) and \(\alpha_2\) peaks, respectively, were 1027 cpm (7086 cpm total) and 266 cpm (1835 cpm total). The \(\alpha_1/\alpha_2\) ratio was 3.9.

The NaCl fraction contained only 2% of the total collagen (Table III) and exhibited an \(\alpha_1\) and \(\alpha_2\) chain ratio of 2.5, typical of type I collagen; no type III molecules were detected (Fig. 7A). The 2.5 M NaCl fraction, containing 70% of the total collagen, was made up of \(\alpha_1\) and \(\alpha_2\) chains in a ratio of 9.5, indicating an identity of type I collagen (Table III, Fig. 7B). Twenty-two percent (22 to 29% in several experiments) of the total collagenous material remained in 2.5 to 5.0 M NaCl fraction (Table III). On CM-cellulose this fraction had both \(\alpha_1\) and \(\alpha_2\) chains, but the ratio was > 8.0 (4.4 to 8.6 in several experiments) (Fig. 7C).

**Table III**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Radioactivity</th>
<th>Collagen radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm (\times 10^{-4})</td>
<td>% of total</td>
</tr>
<tr>
<td>1.5 M NaCl pellet</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>1.5–2.5 M NaCl</td>
<td>38</td>
<td>26</td>
</tr>
<tr>
<td>2.5–5.0 M NaCl</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>5.0 M NaCl super-</td>
<td>77</td>
<td>67</td>
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</tbody>
</table>

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Table III), indicating the presence of excess \( \alpha_1 \) chains. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed that this fraction had a molecular weight of 100,000 (Fig. 8). The 5.0 M NaCl supernatant fraction had 57% of the total radioactivity but did not contain significant amounts of collagen (Table III, Fig. 7D).

In the CM-cellulose procedure employed here, \( \alpha_1[I] \), \( \alpha_1[III] \), and \( \alpha_1[IV] \) chains elute together (4, 6). Therefore, the high \( \alpha_1/\alpha_2 \) ratio of the collagen of the 2.5 to 5.0 M NaCl fraction (which is expected to contain type II collagen) shown in Fig. 7C would indicate the presence of these \( \alpha \) chains. To verify the presence of these \( \alpha \) chains the following experiment was done. Type II and IV chains differ markedly from type I chain in the extent to which lysine is hydroxylated. While 65 and 64% of the lysines are hydroxylated in \( \alpha 1[II] \) and \( \alpha 1[IV] \), respectively (6, 23), only about 15% of these are hydroxylated in \( \alpha 1[I] \). Therefore, the cells were labeled with \( ^{14}C \)lysine and hydroxyllysine of these preparations was measured by amino acid analysis and liquid scintillation counting. As shown in Table IV, the extent of hydroxylation of lysine of the \( \alpha \) chains from the 2.5 to 5.0 M NaCl fraction was not sufficiently high to indicate the presence of type II or type IV collagens.

The \( \alpha \) chains from the 2.5 to 5.0 M NaCl and the 1.5 to 2.5 M NaCl fractions, illustrated in Figs. 7, C and B, were subjected to cyanogen bromide digestion and the peptide pattern was compared by CM-cellulose chromatography and 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis with those of \( \alpha_1[I] \) and \( \alpha_1[II] \) chains. As shown in Fig. 9, the CM-cellulose pattern of the unknown material closely resembled that of the standard human \( \alpha_1[I] \) chains and the \( \alpha_1[I] \) chain from the 1.5 to 2.5 M NaCl fraction. Furthermore, the marker peptide \( \alpha_1[II]CB10 \) (MW, 31,000) (25) was not present in the unknown material. The same conclusions were reached from the electrophoretic experiments shown in Fig. 10.

Collagen of Cell Residue of Fibroblasts from Diseased Gingiva—The cell residue collagen accounted for only 3% of the total synthesized by cells from the diseased tissue. CM-cellulose chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the proteins before and after limited pepsin digestion and reduction, and by DEAE-cellulose chromatography, very similar results have been obtained by others using

**DISCUSSION**

Fibroblasts obtained from human gingiva synthesize quantities of collagenous proteins sufficiently large to permit biochemical characterization. Approximately 60% of the collagenous material remains in the culture medium. Most of this material is procollagen as revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and CM-cellulose chromatography of the proteins before and after limited pepsin digestion and reduction, and by DEAE-cellulose chromatography. Very similar results have been obtained by others using

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**TABLE IV**

<table>
<thead>
<tr>
<th>Hydroxylysine/lysine ratio of ( \alpha 1 ) chains obtained from various NaCl fractions obtained from medium of fibroblasts of diseased gingiva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction</td>
</tr>
<tr>
<td>1.5-2.5 M NaCl</td>
</tr>
<tr>
<td>2.5-5.0 M NaCl</td>
</tr>
<tr>
<td>Human skin collagen, type ( \alpha_1[II] )</td>
</tr>
<tr>
<td>Bovine articular cartilage ( \alpha_1[II] )</td>
</tr>
<tr>
<td>Human glomerular basement membrane collagen ( \alpha_1[II] )</td>
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*From Epstein et al. (24).  
**From Strawich and Nimni (23).  
*** Data of Kefalides (6).
cultured skin fibroblasts (17). The apparent inability of the gingival cells to convert procollagen to collagen may be a consequence of the absence of procollagen peptidase(s) or due to its inaction.

Fibroblasts from normal gingival tissue synthesize both types I and III collagens. Type I collagen, which accounted for 70 to 95% of the total collagenous protein, was characterized by an α1/α2 ratio of 2, and by its typical salt-fractionation behavior and position of migration electrophoretically and chromatographically before and after reduction. Type III collagen accounted for 5 to 30% of the total collagen, and it was identified by its salt fractionation behavior, position of elution from CM-cellulose column and molecular weight before and after reduction. These results are in agreement with previous observations that both type I and III collagens occur in periodontal ligament and gingiva (26, 27) and in normal dog gingiva.

Fibroblasts from periodontally diseased gingiva synthesize collagenous proteins in amounts comparable to normal cells; however, in all experiments, more of the material was found in the medium of the former. Whether or not this observation is important remains unclear since β-aminopropionitrile was present in cultures of both types. Type I collagen was the predominant product of these cells, and type III molecules were not present in quantities sufficient for detection.

An unusual collagenous protein, exhibiting an α1/α2 ratio higher than expected for type I collagen (4.4 to 8.6 in several experiments) appeared in the 2.5 to 5.0 M NaCl fraction of cultures of cells from diseased tissue and accounted for 22 to 79% of the total. This salt fraction is expected to contain type II collagen when it is present. However, the material in this fraction clearly was not type II collagen; the hydroxylysine/lysine ratio was 0.30 instead of 1.87 which is characteristic of type II collagen (23), the cyanogen bromide peptide pattern was similar to that of α1[I] (Fig. 9) and it did not contain α1[II]CB10, the marker peptide for type II collagen (25). Furthermore, the low hydroxylysine content and molecular weight of 100,000 rather than 115,000 expected of α1[IV] (6) would appear to rule out an identity as type IV collagen. The position of elution from CM-cellulose columns and the molecular weight of 100,000 before reduction and alklylation argue against an identity as type III collagen. Likewise, the low amount of α2 chains and the fractionation of the material in the 2.5 to 5.0 M NaCl indicate that it is not type I collagen. The unusual collagen cannot be denatured α1 chains since it was resistant to pepsin digestion and it was precipitable with NaCl, indications of triple helical structure. Neither does the material appear to be reassociated, denatured α1 chains because conditions favoring refolding, such as low pH, temperature fluctuation, and long incubation periods, were not allowed to occur. The material was not found in cultures of normal cells maintained under identical conditions; thus, it is unlikely to be an artificial observation. The data indicate that the unusual collagen has a structure (α1[I]), probably of type I. Support for this conclusion is provided by the similarity of the cyanogen bromide peptide pattern of the unusual material and that of known α1[I] chains (Figs. 9 and 10). Recently Mayne et al. have reported that chondrocytes treated with the mutagen bromodeoxyuridine (28) as well as senescent chondrocytes (29) synthesize a collagen with the structure α1[I], which possesses NaCl fractionation behavior similar to that described above.

The significance of our observation is not clear. In additional experiments not described here we have found the unusual collagen in cells from an additional individual with diseased gingiva and we have failed to detect it in cultures derived from gingiva of another normal individual. Modulation of collagen types is known to occur both in normal growth and in pathologic situations. The proportion of type II molecules in skin decreases with normal growth and development (5). Chondrocytes in osteoarthritic cartilage synthesize type I rather than type II collagen (30). Type III collagen has been found in cirrhotic livers (31, 32) and normal granulation tissue (33), and it is present in inflamed synovial membranes which normally have only type II molecules (34). Although α1[I] collagen has been found in cultures of mutated chondrocytes, it has not been detected in normal or diseased tissue to our knowledge.

The ability of the cells from diseased tissue to produce the unusual collagen and their apparent inability to make type III molecules persist in cultures through numerous cell generations. Thus these cells appear to be genetically different from those obtained from explants of normal gingiva. Mechanisms underlying such a change remain obscure, although several possibilities are apparent. We may have inadvertently detected a previously unknown genetic polymorphism of the collagen locus, although this seems unlikely because the unusual behavior is exhibited by cells from two patients and was not seen in those from two normal individuals. Inflamed gingiva contains a dense plasma cell and neutrophil infiltrate and there is considerable tissue destruction. Conditions of this type may modulate fibroblast synthetic activity, although a change of this type would not be expected to persist in cultures. The most likely mechanism to account for our observations appears to be one of cell selection. The experiments of Martin et al. (39) show that fibroblast populations may be heterogeneous. Chronically inflamed tissues contain toxic substances such as lymphokines and cellular hormones such
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as the prostaglandins; these substances may create selective growth pressures leading to modulation in fibroblast populations.

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Biochemical characterization of collagens synthesized by fibroblasts derived from normal and diseased human gingiva.
A S Narayanan and R C Page


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