TRYPTIC PEPTIDES OBTAINED FROM GELATINS DERIVED FROM NORMAL AND RHEUMATOID ARTHRITIC COLLAGENS
A PRELIMINARY STUDY

by

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The purpose of the present study is to determine whether any differences exist between the primary structure of collagen derived from normal tendon collagen and that of collagen isolated from synovial tissue taken from the knee joints of patients suffering from rheumatoid arthritis. The collagen of tendon is very insoluble and can at present best be studied as the derived gelatin.

Material
Normal tendon was obtained as Achilles tendons from accident cases with no known history of rheumatoid arthritis. Rheumatoid Achilles tendons were not available, but collagenous synovial tissue from the knee joints of patients with severe rheumatoid arthritis, who had undergone arthrodesis, was kindly provided by Mr. R. Tinning of the Rheumatic Diseases Unit, Northern General Hospital, Edinburgh.

Methods
Collagens were prepared from these tissues as follows:
(1) The tissue was cut into small pieces and homogenized in a powerful Waring blender and crude pancreatin was added to degrade the non-collagenous matter, which in the case of the rheumatoid synovial tissue consisted mostly of fat and a mass of soft connective tissue. The weight of collagen processed was not determined because the material was so heterogeneous.
(2) The insoluble collagenous material was centrifuged from the enzyme digestion products and the digestion repeated until the supernatant fluid was clear.
(3) The residual insoluble material was then exhaustively extracted with 0.2M Na2HPO4, followed by water-washing, and was finally extracted with 0.2M acetic acid to isolate any neutral salt-soluble collagen and acetic acid-soluble collagen which might possibly be present.
(4) The residual insoluble collagen was brought to pH 7.0 (pH meter) by addition of 0.1 N NaOH in the presence of 0.2M NaCl and autoclaved at 15 lb/sq. in. for 3 hrs to provide a water-soluble gelatin. This gelatin was isolated by acetone precipitation and dried in a vacuum desiccator; this connective tissue obtained from rheumatoid knee joint yielded 2-3 g. of purified gelatin.

Three rheumatoid and four normal gelatins have so far been examined by tryptic digestion as follows:
100 mg. gelatin was suspended in 10 ml. water and the pH brought to 9 by means of an autotitrator. A solution of trypsin (Armour batch 22190) containing 0.5 mg. enzyme was added and the reaction mixture shaken at 37°C for 18 hrs. A further addition of 0.5 mg. trypsin was followed by shaking for an additional 18 hrs. A small quantity of insoluble material was centrifuged off from the soluble tryptic peptides. The supernatant was brought to pH 6.0 (pH meter) with 0.1 N HCl and desalted on Dowex-50 (Steven and Tristram, 1962).

The tryptic peptides derived from normal and rheumatoid gelatins were then compared by fingerprinting analysis. The peptides were applied to Whatman 3MM paper wetted with pyridine-acetate buffer pH 6.4 (Ingram, 1956), and were subjected to electrophoresis with a potential of 1,000 volts and 45-50 mA. for 2 hrs. After drying, the papers were subjected to ascending chromatography in pyridine: iso-amyl alcohol:water (117:117:100), as described by Baglioni, Ingram, and Sullivan (1961). The peptide spots were detected by dipping the papers in acetone containing 0.5 per cent. ninhydrin followed either by heating in a 60°C. oven for 20 min. or by allowing the peptide spots to stain in a dark cupboard overnight.

Results
A typical finger-print pattern of the soluble tryptic peptides derived from these gelatin is presented in the Figure. This is a general pattern and does not take into account faint spots which appear on over-
loaded chromatograms and are considered to represent only minor constituents. The main difference in the patterns was confined to the peptide spots numbered 1 and 2; these were well-defined strongly-staining spots in normal gelatin but were entirely absent or present in only trace amounts in rheumatoid gelatins.

Discussion

A very small quantity of acetic acid-soluble collagen was obtained from the collagen samples but no soluble collagen could be extracted from normal Achilles tendons. This observation may be associated with the greater catabolic activity of collagen which is known to be present in rheumatoid arthritis (Rubegni, Ravenni, and del Giovane, 1962).

Collagen molecules consist of a tight triple helix made up from three peptide chains; at each end of this rod-like structure is attached a short "end-chain" of random coiled peptide (Hodge, Highberger Deffner, and Schmitt, 1960). Only collagenases attack the triple helical part of the native molecule (Mandl, 1961), whilst the "end-chains" are susceptible to digestion by most proteolytic enzymes (Hodge and others, 1960; Nishihara and Miyata, 1962; Rubin, Pfahl, Speakman, Davison, and Schmitt, 1963). Hodge and others (1960) have demonstrated that the "end-chains" are essential for normal end-to-end polymerization of collagen.

It is probable that the present preparation of collagen from connective tissue by the use of pancreatic trypsin has destroyed part of these "end-chains". It is recognized that the structure of the "end-chains" may well be of great importance in the polymerization process for healthy and diseased connective tissue. The extraction of a native soluble collagen containing "end-chains" from aged tendon has not yet been achieved. With these limitations in mind, it is still of interest to study the structure of the helical part of the collagen molecule, and the purified gelatins described here are derived from this part.

Part of the gelatin remained insoluble after trypsin digestion and this has not been examined in the present work. In the Figure a very complex mixture of tryptic peptides has been partially resolved into about fifty peptide fractions. Further modifications of the finger-printing technique may resolve these peptides more completely and provide more useful information. It is noteworthy that Grassmann, Hannig, Endres, and Riedel (1957) obtained tryptic peptides with a chain average molecular weight equivalent to eighteen amino acid residues. If the molecular weight of a gelatin is taken as approximately 100,000 (Steven and Tristram, 1963) equivalent to 1,000 amino acid residues, the present finding of fifty peptides agrees well with Grassmann's observations.

Conclusions

Two conclusions may be drawn from the present enzyme study:

1. The interior of the collagen molecules in

Figure.—Finger-print map of tryptic peptides obtained from normal and rheumatoid arthritic gelatins. Electrophoresis pH 6.4, 1,000 V for 2 hrs, followed by ascending chromatography in Pyridin: iso-amyl alcohol: water (117, 117, 100) for 18 hr
normal and rheumatoid arthritic tissues is structurally very similar in amino acid sequence.

(2) The tryptic digest of rheumatoid collagen probably differs from that of normal collagen in two peptides, those numbered 1 and 2 in the Figure. In normal collagen tryptic digests, these peptides appear as well-defined spots, whereas in rheumatoid collagen they are entirely absent or are present in only trace amounts. Preliminary results from peptic and collagenase digestion studies of these gelatins also suggest slight differences in peptide structure.

The present study may be taken as an indication of the value of finger-printing techniques for the structural investigation of pathological collagens. It is hoped to confirm these preliminary results by investigating a greater number of derived gelatins and also by increasing the number of techniques available for the preparation of reproducible peptide mixtures suitable for finger-printing analysis.

Summary

Gelatins were prepared from purified collagens obtained from normal tendon and rheumatoid arthritic synovial tissues, and the tryptic digestion products of these gelatins were compared by finger-printing analysis. A close structural similarity was observed. Two peptides were present in normal gelatin which appeared to be entirely absent from (or present in only trace amounts in) the three rheumatoid gelatins so far examined. The usefulness of this type of analysis has been demonstrated for the structural study of pathological collagens.

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Peptides trypsiques obtenus des gélatines dérivées des collagènes normaux et arthritiques rhumatoïdes

RéSUMÉ

On a préparé des gélatines de collagènes purifiés provenant de tissus synoviaux des tendons normaux et arthritiques rhumatoïdes et on a comparé les produits de digestion trypsique de ces gélatines par l’analyse du tableau électrophorétique. On a observé une similarité structurelle étroite. Dans la gélatine normale il y avait deux peptides qui étaient soit absents, soit à peine perceptibles dans les trois gélatines rhumatoïdes examinées jusqu’ici. On avait déjà démontré l’utilité de ce type d’analyse pour étudier la structure des collagènes pathologiques.

Peptidos trópicos obtenidos de gelatinas derivadas de colágenos normales y artríticos reumatoïdes

SUMARIO

Se prepararon gelatinas de colágenos purificados obtenidos de tejidos sinoviales de tendones normales y artríticos reumatoïdes y se compararon los productos de digestión trópica de estas gelatinas por análisis del diseño electroforetico. Se observó una estrecha similaridad de estructura. En la gelatina normal hubo dos peptidos que fueron enteramente ausentes de, o apenas perceptibles en las tres gelatinas reumatoïdes examinadas hasta la fecha. Ya fue demostrada la utilidad de este tipo de análisis para estudiar la estructura de los colágenos patológicos.