Whole tendons of chick embryos synthesize procollagens V which consist of three pro-α chains: pro-α1(V), pro-α1(V) and pro-α2(V). This report shows that while the pro-α1(V) chain is similar to the pro-α1(V) chain in many respects, such as similar but not identical peptide maps, it also distinctly differs from it in size and in other ways. The new chain is denoted as pro-α1 to indicate the relationship. We have failed to see conversion of one chain into the other and they are regarded as variants, although we do not know whether they are different transcripts of one gene or products of two closely related genes. The pro-α(V) chains are assembled into the disulfide-linked homotrimer [pro-α1(V)₃ and the heterotrimer [(pro-α1(V)S₈-pro-α2(V))pro-α1(V)] and a smaller amount of [(pro-α1(V))pro-α2(V)]. The pro-α1(V) chains are processed similarly to the pro-α1(V) by the initial removal of the presumed carboxyl propeptide yielding p-α1(V) and then by reduction in the size of the procollagenous, presumed amino propeptide to yield α1(V). A size difference between the α1(V) and α1(V) series of molecules is demonstrated by velocity sedimentation and by electrophoretic mobility of the reduced molecules. This difference is ascribed to a difference in the size of the peptide sequence because after pepsin digestion the products of both series of molecules are the same size and electrophoresis like α1(V)(pepsin). The carboxyl propeptides of pro-α1(V) and pro-α1(V) are the same size, but the amino propeptide of pro-α1(V) is smaller than that of pro-α1(V). The amino propeptide of pro-α1(V) and p-α1(V) also lacks asparagine-linked complex carbohydrate, which is linked to propeptides of the p-α1(V) and p-α2(V) chains. Differences between the α1(V) and α1(V) series of molecules remain in material synthesized in the presence of tunicamycin.

Primary cultures of tendon cells synthesize procollagen V consisting of the above three chains, but the procollagen V made by cultured tendon sheath synovial cells predominantly contains [(pro-α1(V))pro-α2(V)]. The two cartilage collagen chains 1α and 2α are probably also closely related to them (1). The α3(V) chain has not been described in chick tissues and peptide mapping clearly shows that it is not a variant of the α1(V) chain. In tendon we have now found a procollagen V chain, which we denote as pro-α1(V) (2). This new chain is similar to pro-α1(V) but differs from it at least in size, attachment of complex carbohydrate, and interchange disulfide formation. Furthermore, we were unable to yield interconversion of pro-α1(V) and pro-α1(V) chains and conclude that their differences are not solely due to postribosomal modifications. We suggest that these two polypeptides are either encoded in two closely related genes or are different peptide translation products of a single gene.

Fig. 1 summarizes our limited understanding of individual pro-α1(V) chains and the p-α(V) and α(V) chains into which they are physiologically converted (2-6). Electron microscopy of procollagen shows a 300-nm long thread, corresponding to the 3 x 100,000-dalton collagen helix, with a knob at each end (7). The knobs correspond to large, collagenase-resistant peptide regions. Although the amino and carboxyl ends of procollagens V have not been established, comparison with procollagens I, II, and III suggests that the smaller procollagenous peptides of pro-α1(V) and pro-α1(V) are their carboxyl ends: they are internally disulfide-linked glycopeptides of similar size and the physiological cleavage of all of them is inhibited by 50 mM arginine (3-6). These are cut off when p-collagen is formed, and at the same time any disulfide links between component p-α(V) chains are lost. However, intrachain disulfide links exist in the remaining noncollagenous regions, and correspondingly the electrophoretic mobilities of the denatured, individual p-α(V) chains are slowed on reduction.

To help to understand properties of the p-collagen V chains that were utilized in this study, we describe the pro-α2(V) and p-α2(V) chains, and some noncollagenous "P peptides" which are disulfide-linked to the p-α2(V) chains. The p-α2(V) chain has only one large noncollagenous peptide region, and this may be at the carboxyl or amino end. Its location is under current investigation. This large noncollagenous region is retained in the p-a2(V) chain, but is cut during conversion to α2(V), with a portion remaining attached to the collagen helical sequence of α2(V). Surprisingly, all p-α2(V) chains have more than one copy of an approximately 30,000-dalton noncollagenous peptide, named peptide P, attached by disulfide links (4, 5). The nature and source of the P peptides are unknown; they could be derived from the smaller noncollagenous regions of the pro-a1(V) and pro-a1'(V) chains. The covalent, denatured p-a2(V)-P complex sediments significantly faster than reduced, denatured p-a2(V) chains and also

---

1. In the preliminary publication (2) we referred to the new procollagen V chain of tendon as pro-a1(V) and its products as p-a1(V) and a1(V). The names have been changed to pro-a1'(V), p-a1'(V), and a1'(V) in the present publication.

---

*This research was supported by United States Health Service Grants AM13748 and AG02128, a Biomedical Research Support Grant, and the Laubisch Fund. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
has a correspondingly slower electrophoretic migration (5). The P peptides are lost during physiological, proteolytic conversion to α(V) collagen. All the chains of the final, α(V) collagen derivatives maintain their identities and are similar to, but different from, pro-α1(V) and its derivatives. Pepsin digestion, as used by others to solubilize collagens V from tissues, removes the noncollagenous peptide regions present in α(V) collagen chains to give correspondingly smaller α(V)(pepsin) chains. The electrophoretic mobilities of α1(V)(pepsin) and α(V)(pepsin) are identical, and the heterogeneity of chains which we describe here would not be apparent in materials solubilized by pepsin treatment.

The new chain forms both homo- and heterotrimeric species. Previously, collagen V (pepsin) chains were recovered in various proportions, and this suggested that the three chains of each molecule could be chosen in different combinations. The homotrimer (α1(V)(pepsin))3 and the corresponding (pro-α1(V))3 (6) were isolated from hamster lung cell cultures. The occurrence of (α(V)(pepsin))3 is presently under dispute (9, 10), and a synthetic (α(V)(pepsin))3 was found to be unstable at 37°C (11). The disulfide-linked heterotrimer [pro-α1(V)]3(pro-α2(V))3 was identified (3, 12, 13), and the corresponding molecule [α1(V)(pepsin)α2(V)(pepsin)]3 has been suggested and isolated (9, 10, 14). A heterotrimer of different chains, [α1(V)(pepsin)α2(V)(pepsin)α2(V)(pepsin)]3 was proposed and isolated (10, 14, 15).

A peculiar feature of procollagens V is that, in contrast to procollagens I, II, III, and IV, only a small proportion of the molecules have all three component chains disulfide-linked to each other. Most chains are linked pair-wise, or not at all (4, 6), and the homotrimer (pro-α1(V))3 of hamster lung cells lacks any interchain disulfide links (6). The present work originated in a re-examination of this problem of interchain disulfide linkage. We had found earlier that the degree of disulfide linkage could not be changed significantly by prolonging the residence time of chains within cells (4). A relatively higher proportion of disulfide-linked procollagen V molecules in chick embryo tendon was now found to be due to the new pro-α1(V) chain. We now studied procollagen V synthesis by whole tendon and in two sets of cell cultures separately derived from it: one mostly consisting of the major, tendon matrix fibroblasts and the other of synovial cells which form the tendon covering and sheath (16, 17). Chick embryo crop and blood vessels, which essentially fail to make pro-α1(V) chains, served as controls.

**MATERIALS AND METHODS**

Unless stated otherwise, the details of methods were the same as those which we described before (3–6).

**Isolation of Type V Molecules from Tendons**—Tendons from 18-day chick embryos were excised and preincubated in Dulbecco’s modified Eagle’s medium (DMEM), from which those amino acids were omitted which were later added in radioactive form, and supplemented with 100 μg/ml of ascorbic acid, 64 μg/ml of β-aminopropionitrile, and 0.02 μM Hepes, pH 7.4. The tissue was labeled either with 50 μCi/ml of [5,5₂H]proline and [4,5,5₃H]leucine (ICN Corp.) or [35S]methionine (Amersham Corp.). In a pulse-chase experiment the labeled tendons were rinsed and incubated in DMEM supplemented as above plus 250 μg/ml of cycloheximide (Sigma) and with 0.1 mg/ml of pepsin. The tissues were frozen in liquid N₂ and extracted at 0°C with 1 M NaCl buffer containing protease inhibitors, and the extract was chromatographed on a DEAE-cellulose column as described (3, 5). The native proteins in the concentrated “low salt” or “high salt” fractions were further purified by velocity sedimentation in a Beckman SW 60 rotor at 4°C in a 5–20% sucrose gradient containing 2 M urea, 0.5 M NaCl, 0.05 M Tris-HCl, pH 7.5, and 0.1% Triton X-100 (3). The native procollagen V fractions obtained by sedimentation or the proteins of the high salt fraction were denatured in 6 M urea at 4°C for 30 min and sedimented on a buffered 5–20% sucrose gradient containing 6 M urea at 2°C in a Beckman SW 60 rotor. Fractions were collected and electrophoresed either nonreduced or reduced on SDS-polyacrylamide gels (18) and fluorographed (19). Densitometric measurements were made on an Optronics 1000 densitometer coupled to a VAX 11/780 computer. Sedimentation coefficients were calculated as described (20).

The type V molecules were identified by comparison with known molecules isolated from chick blood vessels (5). The samples in native form were digested with 100 μg/ml of pepsin at 4°C for 18 h in 0.2 M acetic acid and electrophoresed on SDS-4.5% polyacrylamide gels. Specific bands identified on SDS-4% polyacrylamide gels by fluorography were cut out and also digested with V8 protease (Sigma) or clostripain (Millipore), and the resulting peptides were electrophoresed on SDS-10–15% polyacrylamide gels as described (3).

Native type V molecules were immunoprecipitated with antibodies prepared in rabbits against type V collagen extracted by pepsin digestion of cold chick embryos and purified by salt precipitation. The immune complex was formed at 4°C for 18 h and precipitated with Pansorbin (Sigma). The precipitate was sedimented through 1 M sucrose, 50 mM Tris-HCl, pH 7.5, 20 mM EDTA, 0.1% Triton X-100, and the precipitate was washed with this buffer minus sucrose. The proteins were solubilized at 100°C in 1% SDS and 2 M urea. Aliquots of the proteins in the immune precipitate and supernatant were reduced and electrophoresed on a SDS-4.5% polyacrylamide gel and fluorographed.

**Separation of Noncollagenous Peptides**—Type V molecules separated electrophoretically on SDS-4.5% polyacrylamide gels were identified by fluorography after staining with Coomassie blue, and their radioactivity. About 1% of the radioactivity wastractable with buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.6, 7 mM CaCl₂, 0.1% Triton X-100) and bacterial collagenase ( Worthington CLSPA) which had been further purified (21, 22), and incubated at 37°C for 3 h. Then 1% SDS, 10 mM dithiothreitol, and 10% glycerol were added and the gel pieces were heated at 100°C for 3 min and electrophoresed on a SDS-10% polyacrylamide gel (4). The noncollagenous P pellets attached to p-a2(V)-P were identified electrophoretically after reduction of denatured p-a2(V)-P and isolated by velocity sedimentation. This fraction was also digested with bacterial collagenase and the propeptide-P₂ fragment was identified electrophoretically.

**Tendon Cell and Synovial Cell Cultures**—The cells were isolated as described. The abbreviations used are: DMEM, Dulbecco’s modified Eagle’s medium; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate.
described (16) from 17-day chick embryo tendons. These were incubated in phosphate-buffered saline containing 0.1% bacterial collagenase (Worthington CLS) and 0.25% trypsin (Difco) and 0.1% glucose for 20 min at 37 °C to release synovial cells. The residual tendons were rinsed with buffered saline and incubated as above for 2 h at 37 °C to release tendon cells. The cell suspensions were filtered through Nytex gauze, and the cells were washed 3 times in DMEM containing 0.1% fetal calf serum, and 10 mM Hepes buffer, pH 7.4. The tendon cells were allowed to attach to tissue culture plates in this medium for 2 h and the nonattached cells were removed. The cells were then kept for 1 h either with fresh DMEM as above or in DMEM containing 0.22 μg/ml of tunicamycin A₄, kindly donated by Dr. D. Duskin (23). The cultures were then labeled for 18 h with [5'-3H]proline and [4,5'-3H]leucine (each 25 μCi/ml). Some tendon cells were cultured for 3 days until the cells were confluent and labeled as above. The synovial cells were suspended in DMEM containing 5% fetal calf serum. The cells were allowed to attach for 12 h, fresh medium was added, and the cultures were grown to near confluency in 3 days. On the third day the cultures received 25 μg/ml of ascorbate and on the fourth day the cells were labeled for 18 h in medium containing [5'-3H]proline, [4,5'-3H]leucine (25 μCi/ml each), and 25 μg/ml of ascorbate, 64 μg/ml of α-aminopropionitrite, 0.1% fetal calf serum, and 5 mM Hepes buffer, pH 7.4. The type V molecules were isolated from the medium and cell layer as described (6). The DEAE-cellulose chromatography was by stepwise elution of the effluent fraction and the 0.15 M NaCl and 1 M NaCl eluates. The products were examined electrophoretically.

RESULTS

Characterization of Tendon Procollagen V—Chick embryo tendons were incubated in DMEM supplemented with 100 μg/ml of ascorbic acid and 64 μg/ml of β-aminopropionitride in the presence of [3H]leucine and [3H]proline. After a 4-h continuous labeling period the tissue was extracted and the extract was chromatographed on DEAE-cellulose. The effluent fraction contained collagen I and its precursors, the procollagens V eluted predominantly in the low salt fractions and the processed intermediates, p-collagens V, together with the final collagen V were eluted with a high salt buffer (Fig. 2).

The tendon procollagens V were further partially purified in native form by velocity sedimentation. For comparison, procollagen V from blood vessels, consisting of pro-α₁(V) and pro-α₂(V) chains, was sedimented at the same time. The sedimentation coefficients of tendon and blood vessel procollagens V were the same. Electrophoretic analyses of the sediments, reduced samples of tendon procollagens V showed that the three component chains of this procollagen are present in the same proportion in each fraction (Fig. 3). These chains are pro-α₁(V) and pro-α₂(V), and a chain with slightly different electrophoretic mobility, and we refer to this as pro-α₁'(V). Electrophoretic separation of nonreduced aliquots of the same fractions indicated that these procollagens V were present as disulfide-linked trimers, dimers, and pro-α chains (not shown). These were separated after denaturation of the procollagens V by velocity sedimentation, as is shown in Fig. 4. The disulfide-linked trimer fractions consisted almost exclusively of pro-α₁(V) chains, the dimers were composed of equal amounts of pro-α₁'(V) and pro-α₂(V), and the monomers which were not disulfide-linked were predominantly pro-α₁(V) chains and a small amount of pro-α₂(V) chains. From this we conclude that pro-α₁(V) exists as a homotrimer [(pro-α₁(V))-S-S-pro-α₁(V)] and as a heterotrimer consisting of 3 different chains [(pro-α₁'(V))-S-S-pro-α₂(V)]. In addition, smaller amounts of [(pro-α₁(V))-S-S-pro-α₂(V)] may also be present.

Processing of Tendon Procollagen V—When tendons were labeled for 30 min the only radioactive type V chains were the three pro-α(V) chains, and they were labeled in about the same proportions as shown in Fig. 5. However, assembly into disulfide-linked trimers and dimers was incomplete and about half of the chains were present as non-disulfide-linked pro-α₁(V), pro-α₁'(V), and pro-α₂(V). It was not investigated whether these non-disulfide-linked pro-α chains existed as single pro-α chains or whether they were present as triple helically folded molecules. In a pulse-chase experiment, tendons were labeled for 60 min and then transferred to medium containing cycloheximide, and the incubation was continued. Fig. 5 shows that only procollagen V was present at 30 min, but by 60 min of incubation some conversion was seen and thereafter tendon procollagen V was converted first to p-collagen V and then to collagen V. The rates of processing of pro-α₁'(V) and pro-α₁(V) chains were similar.

Characterization of Tendon pro-α₁'(V), p-α₁'(V), and...
$\alpha_1'(V)$—The native p-collagens V sedimented slightly faster than the native collagens V on a sucrose gradient, and in Fig. 6A the electrophoretic analyses of the sedimented, reduced fractions are shown. The p-collagens V consisted of p-$\alpha_1$(V), p-$\alpha_1'$(V), and p-$\alpha_2$(V), and the collagens V consisted of $\alpha_1$(V), $\alpha_1'$(V), and $\alpha_2$(V). The electrophoretic mobilities of reduced p-$\alpha_2$(V) and $\alpha_1'$(V) are closely similar, but the nonreduced $\alpha_1'$(V) chain is clearly distinguishable from the p-$\alpha_2$.P$_n$ molecules, which have a much slower electrophoretic mobility like p-$\alpha_1$(V) (not shown) (4, 5). When the components of this high salt fraction were denatured in 6 M urea at 40 °C for 30 min and sedimented, distinct differences in the sedimentation coefficients of these chains could be seen in Fig. 6B and Table I. Thus, differences in size between the p-$\alpha_1$(V) and p-$\alpha_1'$(V) and $\alpha_1$(V) and $\alpha_1'$(V) molecules are indicated both by electrophoretic mobilities and sedimentation coefficients. The P$_n$ peptides, which are disulfide-linked to the p-$\alpha_2$(V) chain, were also present in tendon p-collagen V and the denatured p-$\alpha_2$(V)-P$_n$ sedimented ahead of all the other chains.

Following pepsin digestion, separated tendon procollagen V and p-collagen V plus collagen V yielded only two electrophoretic bands which were the same as the $\alpha_1$(V)pepsin and $\alpha_2$(V)pepsin isolated from blood vessels or crop. We conclude that the helical portions of the $\alpha_1'$(V) series of molecules are identical or very similar to the $\alpha_1$(V)pepsin chains.

Antibodies prepared against purified collagen V isolated from pepsin digests of decapitated chick embryos precipitated all of the tendon p-collagen V and collagen V, including all of the $\alpha_1'$(V) series of chains (Fig. 7). The results of Fig. 4 show that a significant portion of the total $\alpha_1'$(V) chains in this material exist as homotrimers. Therefore these homotrimers were precipitated by the antibodies, as well as $\alpha_1$(V) chains in heterotrimeric combination with $\alpha_1$(V) and $\alpha_2$(V) chains. Therefore, this new collagen chain is included in the type V group by immunological reaction with antibodies that were made against antigens which met previously set criteria for collagen type V.

Comparisons of the peptide maps obtained by protease digestion of [H]Pro- and [H]Leu-labeled pro-$\alpha_1$(V) and pro-$\alpha_1'$(V) and of p-$\alpha_1$(V) and p-$\alpha_1'$(V) show marked similarities, but some distinct differences can be seen in Fig. 8. The peptide pattern of the $\alpha_2$(V) series is clearly different.

These findings may be interpreted that pro-$\alpha_1$(V) and pro-$\alpha_1'$(V) and their products differ only in their noncollagenous segments. Therefore, we separated the [35S]methionine-labeled or [H]Pro- and [H]Leu-labeled, reduced chains electrophoretically and digested the molecules with bacterial collagenase. The remaining noncollagenous, reduced peptides were then electrophoresed on a SDS-10% polyacrylamide gel together with globular molecular weight standards. Nonre-
Procollagens V

Fig. 6. Velocity sedimentation of tendon p-collagen V and collagen V. A, the native components of the high salt fraction were sedimented on a buffered sucrose gradient at 56,000 rpm for 28 h at 4°C and 34 fractions were collected. B, the denatured molecules were sedimented on a buffered sucrose gradient containing 6 M urea at 56,000 rpm for 24 h at 27°C and 31 fractions were collected. Aliquots of each fraction were reduced and electrophoresed on SDS-4.5% polyacrylamide gels. These fluorograms only show the large, collagenous chains. The smaller "P-peptides" that were released by reduction from the disulfide-linked p-α2-Pn complexes migrated off the gel.

TABLE I
Sedimentation coefficients of denatured p-collagen V and collagen V molecules

The denatured components of the high salt fraction, obtained by DEAE-cellulose chromatography, were sedimented at 56,000 rpm for 28 h at 27°C, 62 fractions were collected, electrophoresed, and fluorographed, the peak positions for each molecule were determined, and the sedimentation coefficients were calculated as described (20).

| Molecule      | S10,W  | Srelativé |
|---------------|--------|-----------|
| p-α1          | 3.03   | 1.06      |
| p-α1'         | 2.94   | 1.03      |
| p-α2-Pn       | 3.87   | 1.35      |
| α1            | 2.86   | 1.00      |
| α1'           | 2.82   | 0.99      |
| α2            | 2.51   | 0.88      |

Produced p-α2-Pn complex was isolated in denatured form by velocity sedimentation (as in Fig. 6B). This fraction was then either reduced to release and identify the P peptides, or the material was digested first with bacterial collagenase and then the products were analyzed electrophoretically before and after reduction on SDS-10% polyacrylamide gels. Table II summarizes the findings and the diagram in Fig. 1 depicts the differences between the three series of molecules. Since these noncollagenous peptides are glycosylated and sulfated (24), the relative molecular sizes shown are only approximate. The noncollagenous peptides, presumed to be the carboxyl propeptides, cleaved from pro-α1(V) and pro-α1'(V) during the first step of processing, have the same electrophoretic mobilities. The peptides presumed to be the amino propeptides of pro-α1(V) and p-α1(V) are larger than the corresponding peptides of pro-α1'(V) and p-α1'(V). In the next processing step these are cleaved and the remaining noncollagenous peptide of the α1(V) chain is larger than that of the α1'(V) chain.

Although the data given above suggest distinct differences in size of the complete chains, post-translational addition of complex carbohydrate may affect the electrophoretic mobilities of the noncollagenous peptides markedly. The inhibition of this modification of procollagen I with tunicamycin has been analyzed in detail by Duskin and co-workers (23, 25). Procollagen I synthesis was not affected markedly in the cell cultures examined, but processing of pC-collagen I to collagen I was decreased, presumably due to a deficiency of the carboxyl-procollagen peptidase (25). Using freshly isolated tendon cells in culture, which were labeled with [3H]Pro and [3H]Leu for 12 h, we found that procollagen I was almost completely converted to an equal amount of pC-collagen I and collagen I in the culture medium in control cultures. In the presence of tunicamycin A₅ (0.22 µg/ml), procollagen I processing was reduced and pC-collagen I accumulated in the culture medium while only a small amount of collagen I was present. The electrophoretic mobilities of pro-α1(I) and pro-α2(I) and pC-α1(I) and pC-α2(I) were increased when complex carbohydrate addition was prevented by tunicamycin. The synthesis of type V procollagens and the processing to p-collagen V was nearly the same in the presence or absence of tunicamycin. Under the specific culture conditions no processing to final collagen V occurred. The p-α1(V) and p-α2(V) chains showed altered electrophoretic mobilities in cultures treated with tunicamycin, but no change was seen in the mobilities of the p-α1'(V) chains as is shown in Fig. 9. However, the mobility of the pro-α1'(V) chains was altered. Comparisons of the electrophoretic mobilities of the noncollagenous peptides of these molecules from control and tunicamycin-treated cultures are given in Table III. The presumed amino propeptides of either pro-α1'(V) or p-α1'(V) did not show differences, while the corresponding propeptide of p-α1(V) and the noncollagenous peptide of p-α2(V) had greater...
Procollagens V

**TABLE II**

Relative electrophoretic mobilities of the noncollagenous peptides

| Source | Larger peptides | Smaller peptides | P-peptide |
|--------|----------------|------------------|-----------|
| A      |                |                  |           |
| pro-α1(V) reduced | 0.25 | 0.52 |          |
| pro-α1′(V) reduced | 0.31 | 0.52 |          |
| p-α1(V) reduced | 0.25 | |          |
| p-α1′(V) reduced | 0.31 | |          |
| α1(V) reduced | 0.44 | |          |
| α1′(V) reduced | 0.54 | |          |
| B      |                |                  |           |
| p-α2 + P peptides reduced | 0.47 | | 0.58 |
| p-α2-Pα nonreduced | 0.09 | | |

**Fig. 7.** Immune precipitation of native p-collagen V and collagen V. The labeled molecules were separated by DEAE-cellulose chromatography as in Fig. 2, and the components of the high salt fraction were precipitated with anti-collagen V (pepsin) antibodies. The fluorogram of the type V molecules present in the immune precipitates (Ppt) and supernatants (S) is shown.

**Fig. 8.** Peptide maps of type V molecules treated with *Staphylococcus* V8 protease or clostripain. The reduced molecules were separated electrophoretically and then digested with the enzymes indicated, and the products were electrophoresed and fluorograms are shown.

mobilities when addition of complex carbohydrate was prevented. The "carboxyl propeptide" of pro-α1′(V), on the other hand, contains complex carbohydrate as evidenced by increased mobility of this peptide derived from pro-α1′(V) of a tunicamycin-treated culture.

**Site of Synthesis of the New Type V Collagens**—Tendon cells consist primarily of tendon fibroblasts and of an outer sheath of tendon synovial cells. These two cell types can be separated by differential attachment to tissue culture plates (16). The tendon cells attach within 2 h in the presence of 0.1% fetal calf serum, and in the presence of 25 μg/ml of ascorbic acid these cells synthesize collagen at the same rate as in the whole tissue (26). During the first 24 h of culture gene expression does not appear to be altered. These tendon fibroblasts make types I and V collagen, but no type III collagen, while the sheath cells make type III collagen as well (16, 17). Tendon fibroblasts maintained in tissue culture for a few days initiate type III procollagen synthesis (27). Under the conditions used in our experiments the tendon fibroblasts synthesized types I and V procollagens, but no type III collagen was detectable when the medium proteins were digested with pepsin and the nonreduced molecules were electrophoresed. These tendon fibroblasts released most of the type V...
molecules into the medium and processing to p-collagen V occurred (Fig. 9). Tendon cell cultures grown for 3 days and then labeled also synthesized pro-al(V), pro-al(V), and pro-al'(V) chains. Tendon synovial cells are readily released from whole tendons with bacterial collagenase and trypsin, and these cells attach to tissue culture plates only in medium containing 5% fetal calf serum. These cells were cultured for 3 days to obtain sufficient cells for labeling. These cells also synthesized type V molecules. Most of the type V molecules were deposited in the cell layer, some pro-al(V) and pro-al(V) and mostly al(V) and al(V) were present, and only a trace of p-al'(V) and al'(V) were seen. The latter could have been made by contaminating tendon cells. The synovial cells also made type III procollagen (as confirmed by pepsin digestion of the medium proteins). This could be a specific cell type marker expressed predominantly by the tendon matrix fibroblasts and probably to a lesser extent or not at all by the surrounding tendon sheath synovial fibroblasts.

**DISCUSSION**

There are at least three differences between the pro-al(V) and the pro-al'(V) chains: 1) the mass of the noncollagenous region near the presumed amino end of the collagen helical portion, 2) attachment of complex carbohydrate to the presumed amino noncollagenous region, and 3) the propensity to form disulfide links with other members of the same triple-chained molecule.

The masses of the pro-al'(V), p-al'(V), and al'(V) chains are each smaller than those of, respectively, the pro-al(V), p-al(V), and al(V) chains. The different electrophoretic behavior of collagenous and noncollagenous peptides and the influence of glycosylation only allow nominal estimation of molecular masses (Fig. 1). However, the same sequence of relative molecular sizes is indicated by the electrophoretic mobilities and the sedimentation velocities of the denatured chains. This same sequence of relative electrophoretic mobilities is maintained when attachment of complex carbohydrate is prevented by tunicamycin, even though some individual electrophoretic mobilities are changed. Therefore, there are underlying differences which are independent of attachment of complex carbohydrate. After digestion with bacterial collagenase, the equivalent differences of electrophoretic mobilities are shown by the separated, presumed amino noncollagenous peptides. In contrast, the carboxyl propeptides of pro-al(V) and pro-al'(V) have the same mobility. As al(V)(pepsin) and al'(V)(pepsin) also have identical electrophoretic mobilities, we conclude that one key difference between the pro-al(V) and pro-al'(V) chains is in the masses of those parts of the presumed amino noncollagenous peptide regions which remain attached in the al(V) and al'(V) chains, i.e. the masses of their telopeptides. Differences in the peptide maps produced by protease digestion are consistent with this view. In addition, our unpublished experiments show that some tyrosine residues of these noncollagenous peptides of pro-al(V), al(V), p-al'(V), and al'(V) are sulfated. When [35S]sulfate-labeled molecules were digested with *Staphylococcus aureus* V8 protease, the patterns of radioactive peptides obtained from the p-al(V) and al(V) series were very different from those of the p-al'(V) and al'(V) chains. This provides further evidence that the noncollagenous segments of al(V) and al'(V) are different.

Purified tunicamycin caused closely similar modulations of procollagen I synthesis and processing by tendon fibroblasts to those described by Duskin and co-workers (23, 25), who verified the action of the drug by mannose incorporation measurements. As these cells simultaneously made procollagens V, the effect of tunicamycin on the electrophoretic mobilities of the procollagen V chains can be interpreted as affecting attachment of complex carbohydrate. Tunicamycin changed the electrophoretic mobility of pro-al'(V) but not of pro-al'(V) chains (Fig. 9). We conclude both that complex carbohydrate is attached to the carboxyl propeptide and that, necessarily, the drug had equal access to the biosynthetic paths of pro-al(V) and pro-al'(V). Interestingly, complex carbohydrate is attached to the noncollagenous peptides of both p-al(V) and p-al'(V) chains (Table III). At present we do not know whether complex carbohydrate is attached to the noncollagenous regions of al(V) and al'(V) chains.

Our conclusion that most of the pro-al'(V) chains are disulfide-linked to neighbors within the three-chained molecules, in contrast to pro-al(V) chains which are mostly unlinked, is based on a range of experiments of which only one is illustrated in Fig. 4. The sedimentation velocity of these materials under native conditions indicated that they consisted of individual three-chained molecules, and, therefore, the disulfide-linked dimers and trimers represent their constituents. Fig. 4 strongly indicates the existence of heterotrimetric molecules which contain all three chains. Denaturation yielded mostly individual pro-al'(V) chains in the necessary amount to provide the third chain required by the disulfide-linked pairs of pro-al'(V) and pro-al(V) chains. When electrophoretograms of denatured procollagen V are interpreted, it is essential to be aware of the small decreases in electrophoretic mobility of individual chains that accompany reduction of the disulfide-links which hold folds within one chain together. As a consequence, reduced pro-al'(V) chains have almost the same mobility as nonreduced pro-al(V) chains. Complete reduction before analysis avoids this problem.

We have not succeeded in separating the central, collagenous regions of the al(V) and al'(V) chains after pepsin digestion of the mixture of native, helical molecules. Cyanogen bromide digest maps of unfraccionated, labeled collagen V(pepsin) from tendon and from blood vessels, which do not contain the al'(V) chain, were very similar. Because of the possibilities of incomplete digestion and other effects, we excluded these experiments on mixtures of collagen V(pepsin) chains from the present considerations. However, together with the protease peptide maps, the evidence clearly indicates that the collagenous portion of the pro-al'(V) chain and its

**TABLE III**

| Source | Large (amino) peptide | Small (carboxyl) peptide |
|--------|-----------------------|-------------------------|
| Control | +Tunicamycin | 3.45 | 4.05 |
| pro-al'(V) | 1.7 | 1.7 |
| p-al'(V) | 1.7 | 1.7 |
| pro-al(V) | 1.5 | 1.55 |
| al(V) | 1.5 | 1.55 |

The peptide patterns of human \( \alpha(V)(\text{pepsin}) \) and \( \alpha(V)(\text{pepsin}) \) have not been described in chick tissues. The cyanogen bromide derivatives is similar to that of the pro-\( \alpha(V) \) and unlike the chick.

The similarities of the pro-\( \alpha(V) \) and pro-\( \alpha(V) \) chains and their derivatives raise the possibility that they are different, secondary modifications of a single polypeptide. We looked for evidence towards this, failed to find it, and conclude that they represent different products of ribosomal peptide and their derivatives raise the possibility that they are different products of ribosomal peptide.

We suggest that there must be differences in the precursor polypeptide. The existence of the heterotramer molecules \( \{ \text{pro-}\alpha'(V) - S-S - \text{pro-}\alpha(V) \} \) necessitates that both the pro-\( \alpha'(V) \) and the pro-\( \alpha(V) \) chains are synthesized simultaneously within the same individual cells and follow a common intracytoplasmic path of synthesis and transport.

The hypothesis of a single polypeptide precursor for both chains would require at least the following secondary modifications to some chains, but not to others: 1) attachment of complex carbohydrate to the "amino" noncollagenous region of the pro-\( \alpha(V) \) chain, but not to the pro-\( \alpha'(V) \) chain (even though complex carbohydrate is attached to the carboxyl propeptides of both chains); 2) additional increase of mass of this amino noncollagenous region of pro-\( \alpha(V) \), separately from attachment of complex carbohydrate, by some unspecified process; 3) by some unspecified process assuring that interchain disulfide links are made by the pro-\( \alpha'(V) \) chains at a location at least 100,000 daltons away, beyond the other end of the central collagen peptide sequence, from the first two modifications. It seems improbable that three such effects would all be exercised on one copy of a polypeptide and not on another identical one passing along the same intracytoplasmic path.

We suggest that there must be differences in the precursor polypeptides themselves. These differences could be due to different genes, to different transcripts of one gene, or to different splicing combinations of one set of transcripts into two different mRNAs.

The pro-\( \alpha'(V) \) chains may be a specialization of the tendon matrix cells. They are not made by the smooth muscle cells of crop and blood vessels and probably also not by synovial tendon cells. The role of the substantial noncollagenous regions which seem to remain in the \( \alpha(V) \) chains after physiological processing is unknown, and it would be interesting if there were tissue-specific modification of these regions.

Acknowledgments—We thank Homero Dewes for the CNBr peptide analyses of the \( \alpha(V)(\text{pepsin}) \) chains obtained from chick blood vessels and tendons.

REFERENCES

1. Bornstein, P., and Sage, H. (1980) Annu. Rev. Biochem. 49, 957
2. Fessler, L. I., Shigaki, N., and Fessler, J. H. (1983) Fed. Proc. 42, 1886
3. Kumamoto, C. A., and Fessler, J. H. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 6454
4. Kumamoto, C. A., and Fessler, J. H. (1981) J. Biol. Chem. 256, 7053
5. Fessler, L. I., Kumamoto, C. A., Meis, M. E., and Fessler, J. H. (1981) J. Biol. Chem. 256, 9640
6. Fessler, L. I., Robinson, W. J., and Fessler, J. H. (1981) J. Biol. Chem. 256, 9646
7. Bächinger, H. P., Doege, K. J., Petschek, J. P., Fessler, L. I., and Fessler, J. H. (1982) J. Biol. Chem. 257, 14590
8. Haralson, M. A., Mitchell, W. R., Kresina, T. F., Gay, R., and Miller, E. J. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5208
9. Madri, J. A., Foellmer, H. G., and Furthmayer, H. (1982) Collagen Relat. Res. 2, 19-29
10. van der Rest, M., and Niybhi, C. (1984) J. Cell. Biochem. 28, 281
11. Bentz, H., Bächinger, H. P., Glanville, R., and Kühn, K. (1978) Eur. J. Biochem. 92, 563
12. Narayanan, A. S., and Page, R. C. (1983) J. Biol. Chem. 258, 11694
13. Alitalo, K., Myllyla, R., Sage, H., Pritzl, P., Vaheeri, A., and Bornstein, P. (1982) J. Biol. Chem. 257, 9016
14. Sage, H., Pritzl, P., and Bornstein, P. (1981) Biochemistry 20, 3778
15. Rhodes, R., and Miller, E. J. (1981) Collagen Relat. Res. 1, 337
16. Riederer-Henderson, M. A., Gaugau, A., Olson, L., Robertson, C., and Greenlee, T. K. (1981) J. Cell Biol. 91, 149a
17. Riederer-Henderson, M. A., Gaugau, A., Olson, L., Robertson, C., and Greenlee, T. K., Jr. (1983) In Vitro 19, 127
18. Laemmli, U. K. (1970) Nature 227, 680
19. Bonner, R. A., and Mills, A. D. (1975) Eur. J. Biochem. 56, 335
20. Fessler, L. I., and Fessler, J. H. (1974) J. Biol. Chem. 249, 7637
21. Peterkofsky, B., and Diegelman, R. (1981) Biochemistry 10, 988
22. Elens, P., Berger, K., Golditch, M., and Schmeir, M. (1973) Anal. Biochem. 53, 315
23. Duksin, D., and Mahoney, W. C. (1982) J. Biol. Chem. 257, 3105
24. Fessler, L. I., Chapin, S., Brosh, S., and Fessler, J. H. (1985) Fed. Proc. 44, 1064
25. Duksin, D., and Bornstein, P. (1977) J. Biol. Chem. 252, 965
26. Schwartz, R. I., Farson, D. A., and Bissell, M. J. (1979) In Vitro 15, 941
27. Herrmann, H., Desau, W., Fessler, L. I., and von der Mark, K. (1980) Eur. J. Biochem. 105, 63