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Characterization of Collagen Synthesized by Normal and Chemically Transformed Rat Liver Epithelial Cell Lines[†]

Barbara D. Smith* and Richard Niles

ABSTRACT: A rat liver epithelial cell line (K16) and a 2-[N-(acetoxyacetyl)amino]fluorine-transformed K16 cell line (W8) [Weinstein, I. B., Yamaguchi, R., Gerbert, R., & Kaighn, M. E. (1975) *In Vitro* 11, 130-141] synthesize and secrete similar quantities of collagenous proteins. The transformed cells produce more total protein so that the percent collagen produced is decreased compared to that of the parent cell line. The type of collagen produced by the transformants differed from that of the parent cell line. After limited pepsin digestion, the pepsin-resistant collagen molecules synthesized by K16 cells precipitated at 2.6 M NaCl and contained both $\alpha_1(I)$ and α_2 chains in a 2.5:1 ratio. The collagen synthesized

by the W8 cells precipitated at higher salt concentrations, and no pepsin-resistant α_2 chains could be demonstrated by CM-cellulose chromatography or gel electrophoresis. The cyanogen bromide peptides of the W8 α_1 chains did not cochromatograph with type I rat skin collagen cyanogen bromide peptides on CM-cellulose chromatography, whereas the α_1 chains of the K16 cells did cochromatograph. The conversion of procollagen to collagen was also decreased in the transformant cells. Therefore, the chemical transformation of K16 produced transformants with increased protein synthesis and altered collagen metabolism.

Many studies (Green & Goldberg, 1965; Green et al., 1966; Hata & Peterkofsky, 1977; Kamine & Rubin, 1977; Levinson et al., 1975; Temin, 1965; Arbogast et al., 1977; Sundarraj & Church, 1978; Adams et al., 1977; Rowe et al., 1978) have established that collagen synthesis by fibroblasts is affected by viral transformation. Chicken fibroblasts infected with Rous sarcoma virus have decreased the overall synthesis of collagen (Levinson et al., 1975; Kamine & Rubin, 1977; Arbogast et al., 1977; Rowe et al., 1978; Adams et al., 1977) as well as decreased the processing of collagen into the extracellular matrix (Arbogast et al., 1977). Using temperature-sensitive virus mutants, it has been shown that the changes in collagen synthesis are a result of transformation and not virus infection (Kamine & Rubin, 1977; Arbogast et al., 1977). Furthermore, the decreased collagen production is accompanied by a decrease in translatable collagen mRNA (Adams et al., 1977; Rowe et al., 1978) and in collagen mRNA sequences (Rowe et al., 1978).

In mammalian systems, a similar but not as dramatic decrease in collagen synthesis has been observed in virally infected cells (Green & Goldberg, 1965; Hata & Peterkofsky, 1977) as well as alterations in hydroxylation and procollagen conversion to collagen (Sundarraj & Church, 1978). In addition, two studies (Hata & Peterkofsky, 1977; Smith et al., 1979) of chemically transformed fibroblasts show that collagen synthesis decreases.

Collagen is a family of genetically distinct molecules. At least four types of collagen have been described. The most abundant and well described collagen, designated type I, is composed of two $\alpha_1(I)$ chains and one dissimilar collagen chain, α_2 . Under certain circumstances $\alpha_1(I)$ chains have been demonstrated to form a triple helical molecule referred to as $\alpha_1(I)$ trimer (Mayne et al., 1975; Moro & Smith, 1977). Other collagen molecules such as type II collagen, found

primarily in cartilage, and type III collagen, widely distributed in blood vessels and reticular fibers, are composed of three similar α chains with unique primary sequences characteristic of the collagen type. Basement membranes contain a separate type IV collagen which may be composed of only one α chain (Kefalides, 1971) or several component α chains. Recent evidence suggests that there are at least two chains which can be cleaved by pepsin (Schwartz & Veis, 1978; Timpl et al., 1979; Kresina & Miller, 1979; Sage et al., 1979). Other collagen chains, probably not of basement membrane origin, have been described and designated A and B chains (Burgeson et al., 1976; Chung et al., 1976) and C chains (Brown et al., 1978). The exact molecular form and significance of these chains are not clear at the moment. Investigations by Hata & Peterkofsky (1977) suggest that 3T3 cells synthesize different types of collagen when transformed by virus or a chemical. Therefore, transformation may involve a switch in collagen type synthesized as well as a quantitative change in synthesis.

Most human tumors are carcinomas derived from epithelial cells, yet little information regarding collagen synthesis is available on transformed epithelial-like cells. It is known that rat liver epithelial cells in culture produce collagenous proteins (Sakakibara et al., 1977, 1978; Berman et al., 1978). It is of interest to see if epithelial cells as well as fibroblasts produce decreased levels of collagen after transformation. In the present paper we describe the amount and type of collagen produced by a rat liver epithelial cell line, K16, compared to the collagen produced by a chemically transformed tumorigenic cell line, W8, derived from K16 (Weinstein et al., 1975).

Methods and Materials

Culture Techniques. Rat liver epithelial cells (K16) and chemically transformed cells (W8) were kindly provided by Dr. I. B. Weinstein. [Weinstein et al. (1975) treated the rat liver epithelial cell line K16 with a single exposure to 0.05 mg/mL 2-[N-(acetoxyacetyl)amino]fluorene.] For this study, low passage cells were grown in Dulbecco's modified Eagle's medium (DME medium) with 10% serum, 1% penicillin G-streptomycin sulfate, and 1% glutamine. Cells were subcultured by dissociating the cells with trypsin-EDTA solution (Gibco) for 5 min at 37 °C. Usually 200 000-600 000 cells

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were plated on a T75 flask. The cells reached confluence in 5–7 days.

Radioactive Labeling. Cells were always radioactively labeled just after reaching confluence. For studies determining the amounts of collagen synthesized, cells were preincubated in Eagle's medium without glutamine or serum but in the presence of ascorbic acid (100 µg/mL) and β -aminopropionitrile (BAPN) (100 µg/mL) and then labeled for 6 h by adding 1 µCi/mL [^{14}C]proline. For studies involving collagen isolation, cells were preincubated in glycine, serum, and glutamine deficient DME medium containing 100 µg/mL ascorbic acid and 100 µg/mL BAPN. Either 100 µCi/mL [^3H]glycine and [^3H]proline or 20 µCi/mL [^{14}C]glycine and [^{14}C]proline was added for 24 h. Radioactive media were removed, and protease inhibitors were added so that the final concentration was 0.02 M EDTA, 10 µM phenylmethanesulfonyl fluoride (PMSF), and 1 mM *p*-(chloromercu)-benzoate (PCMB) in 0.05 M Tris, pH 7.4 (Smith et al., 1977). Cell layers were washed with saline-containing inhibitors added to the media and centrifuged (400g for 15 min). The resulting supernatant was stored frozen, and the precipitate was added to the cell layer. The cell layers were scraped in 0.5 N acetic acid and stored frozen.

Quantitation of Collagen. Medium and cell layers were dialyzed against water, lyophilized, and weighed. Samples were brought up in 1 mL of 0.15 N NaCl containing (2 mg/mL) albumin. Proteins were precipitated by 10% trichloroacetic acid (Cl_3AcOH) at 4 °C. The precipitates, after several washes, were collagenase (Advanced Biofactors, Form III) digested and assayed in duplicate as previously described by Peterkofsky & Diegelmann (1971). Duplicate flasks set up at the same time were used for cell counts and DNA determinations (Burton, 1956).

Other media and cell layers, treated the same as above, were flushed with nitrogen and hydrolyzed in 6 N HCl for 24 h at 104 °C. Amino acid analyses were performed on a Jeolco amino acid analyzer equipped with a split-stream device. The radioactivity in 1-mL aliquots from 1.2-mL fractions was counted on a Packard Tri-Carb scintillation counter. Duplicate flasks were grown at the same time for DNA determinations (Burton, 1956).

Collagen Isolations and Salt Fractionations. In all experiments cells layers and media were analyzed separately. Cell layers were homogenized by a polytron and extracted in 0.5 N acetic acid overnight at 4 °C. The homogenate was centrifuged at 15000g for 20 min. The supernatant and precipitate were then desalted by dialysis, lyophilized, and analyzed by gel electrophoresis before and after collagenase treatments. The precipitate was pepsin treated (100 µg/mL pepsin) for 16 h at 4 °C, desalted, lyophilized, and analyzed by gel electrophoresis. In other experiments the cell layer and added carrier lathyritic rat skin collagen (1–5 mg) were homogenized in 0.5 N acetic acid, pepsin treated (100 µg/mL) for 16 h at 4 °C, and centrifuged at 15000g for 20 min. The pepsin in the extract was inactivated by adding Tris to a concentration of 0.05 M and adjusting the pH to 8.5 with NaOH. Type I collagen was precipitated by dialyzing the pepsin extract against 2.6 M NaCl and 0.05 M Tris, pH 7.6. After centrifugation (15000g for 20 min) the precipitate was redissolved in 10 mL of 0.05 M acetic acid. The supernatant with additional carrier rat skin collagen was dialyzed against 4.4 M NaCl and 0.05 Tris, pH 7.4, to precipitate any remaining collagen.

Aliquots of media with inhibitors were desalted by Bio-Gel P-2 columns in 1% acetic acid, frozen immediately in dry ice,

and lyophilized. The rest of the sample was dialyzed against 0.5 N acetic acid, pepsin treated, and salt fractionated as described for the cell layers.

CM-cellulose Chromatography of Collagen α Chains. Further fractionation of collagen α chains was carried out by dialyzing radioactive samples with carrier rat skin type I collagen against 0.05 M sodium acetate buffer, pH 4.8, overnight. Samples were denatured by heating at 41 °C for 10 min and applied to a (2.2 × 12.5 cm) CM-cellulose column preequilibrated with deaerated 0.05 M sodium acetate buffer, pH 4.8. The column maintained at 41 °C was then washed with this buffer, and the bound collagen chains were removed by a linear gradient of 0–0.1 M NaCl over a total volume of 200 mL as described by Piez et al. (1963). Radioactivity in aliquots from each 5-mL fraction was counted in a Beckman (LS-230) scintillation counter. Radioactive proteins eluted off the columns were desalted either by Bio-Rad P-2 columns in 0.1% acetic acid or by exhaustive dialysis against water. Samples were then lyophilized for further analysis.

Cyanogen Bromide Digestion and Peptide Mapping. Lyophilized samples were dissolved in 1 mL of 70% formic acid without additional carriers, warmed to 50 °C, and flushed with nitrogen. Approximately 5 mg of cyanogen bromide was added. The tubes were immediately sealed and incubated at 30 °C for 4 h. After digestion the samples were diluted and lyophilized several times to remove excess cyanogen bromide.

The peptides were analyzed by CM-cellulose chromatography and by gel electrophoresis. Lyophilized peptides and 1–5 mg of added peptides from type I rat skin collagen were dissolved in 10 mL of 0.02 M NaCl and 0.02 M sodium formate, pH 3.8 buffer. The peptides were resolved, as described previously (Lichtenstein et al., 1975), with a linear 0.02–0.14 M NaCl gradient using an LKB gradient maker. Total elution volume was 200 mL.

Polyacrylamide Slab Gel Electrophoresis and Autoradiography. Proteins and cyanogen bromide peptides were examined by sodium dodecyl sulfate (NaDODSO_4) slab gel electrophoresis according to the method of Laemmli (1970). A 5 or 7.5% separating gel was used for whole α chains, and a 10% separating gel was used for cyanogen bromide peptide maps. Samples were dissolved in NaDODSO_4 -urea sample buffer with and without dithiothreitol (50 mM) and heated at 90 °C for 5 min before application to wells. Gels were stained with Coomassie blue R250 in acetic acid and methanol. Gels labeled with tritium were permeated with dimethyl sulfoxide and 2,5-diphenyloxazole for fluorography, dried under vacuum, and exposed to X-ray film by the methods of Bonner & Laskey (1974). Radioactive chick type I collagen was isolated by acid extraction and salt precipitations from 24-h [^{14}C]proline-pulsed 16-day-old chick calveria and was used as a radioactive standard for gel electrophoresis.

Results

Collagen Synthesis by Rat Liver Epithelial Cell Lines. Both the parent rat liver epithelial cell line, K16, and the chemically transformed cell line, W8, incorporated proline into collagenous protein. The uptake of proline into total protein was linear up to 10 h (data not shown). The collagens accumulated in the cell layer and media to about the same extent after 6 h of labeling (Tables I and II). Slightly more radioactive proline was incorporated into the transformed cell collagen as judged by collagenase susceptibility and hydroxyproline content. However, the amount of proline incorporated into non-collagenase-sensitive protein increased dramatically with transformation, especially in the cell layer. The actual amount of protein per DNA or cell number, determined by the Lowry

Table I: [¹⁴C]Proline Incorporated into Cl₃AcOH-Precipitable and Collagenase-Sensitive Protein by K16 and W8 during a 6-h Pulse^a

	total protein ^b		collagen ^c		% radioact in collagen ^d	
	K16	W8	K16	W8	K16	W8
total	295	3310	60	95	4.5	0.5
cell layer	250	3100	30	50	2.5	0.3
media	45	210	30	45	27.0	4.8

^a Each value is the average of three separate collagenase experiments performed in triplicate. DNA/flask was 300–500 µg.

^b cpm of [¹⁴C]proline per µg of DNA in the Cl₃AcOH precipitate.

^c cpm of [¹⁴C]proline in collagenase-sensitive protein per µg of DNA.

^d % radioactivity in collagen = (collagen cpm × 100)/[5.4(noncollagen cpm) + collagen cpm]. This equation corrects for the fact that collagen contains more proline than other proteins (Peterkofsky, 1972).

Table II: [¹⁴C]Proline and [¹⁴C]Hydroxyproline Content of Proteins Synthesized by K16 and W8 Rat Liver Epithelial Cells during a 6-h Pulse^a

	cpm of [¹⁴ C]proline per µg of DNA		cpm of [¹⁴ C]hydroxyproline per µg of DNA		% radioact in collagen ^b	
	K16	W8	K16	W8	K16	W8
total	200	2400	32	42	6.6	0.7
cell layer	170	2230	15	15	3.4	0.25
media	30	170	17	27	32.6	6.5

^a Each value is an average of amino acid determinations on three separate flasks. DNA/flask was 300–500 µg. ^b % radioactivity in collagen = [2([¹⁴C]hydroxyproline cpm) × 100]/[5.4([¹⁴C]proline cpm – [¹⁴C]hydroxyproline cpm) + 2([¹⁴C]hydroxyproline cpm)]. This equation, derived to compare amino acid data to those of collagenase digest assays, is based on the fact that collagen contains approximately equal amounts of proline and hydroxyproline. Therefore, the amount of cpm in the non-collagen protein is proline cpm minus hydroxyproline cpm (Smith et al., 1979).

method, was 4 times greater for W8 than for K16 cells (data not shown). The media from the K16 line contained a very high percent of collagen, higher than for many fibroblastic cell lines. After long-term cultures (>80 passages) this percent collagen decreased for the K16 only (data not shown). The percent collagen, as determined by hydroxyproline content, was comparable to the percent collagen determined by the collagenase assay.

Acid extraction of the cell layer solubilized all the collagen. The pellet remaining after acid extraction contained no collagenase-sensitive proteins on a 5% polyacrylamide gel (not shown), and additional pepsin treatment of the pellet did not solubilize additional collagenous proteins. Figure 1 shows the fluorogram of the acid-extracted cell layers electrophoresed on NaDODSO₄-5% polyacrylamide gels with and without collagenase treatment. The K16 cell layer contained collagenase-sensitive $\alpha 1$ and $\alpha 2$ chains that migrated exactly with both collagen standards (radioactive chick calvaria type I chains and lathyritic rat skin type I collagen). However, the W8 cell layer did not have collagenase-sensitive proteins migrating with $\alpha 2$ chains. The fastest migrating α chain was found in the position of pN $\alpha 2$ rather than $\alpha 2$. Another collagenase-sensitive band appeared in the pro α chain region. These three collagenase-sensitive protein bands in the W8 cell layer remained after pepsin treatment whereas only $\alpha 1$ and $\alpha 2$ were observed for the K16 cell layers after pepsin treatment.

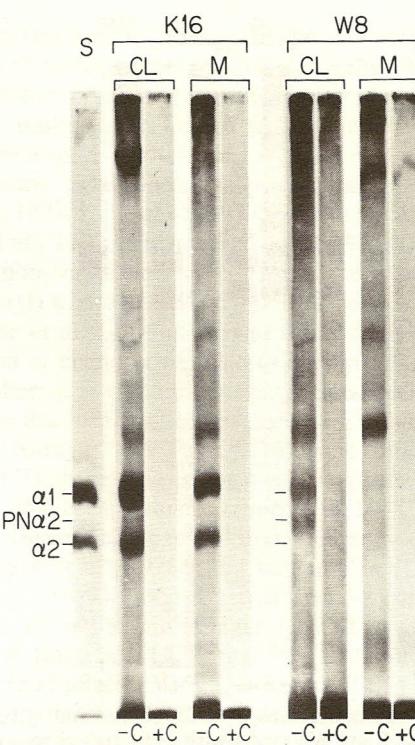


FIGURE 1: Autoradiograms of [¹⁴C]proline- and -glycine-labeled proteins in K16 and W8 acid-extracted cell layers (CL) and media (M) with (+C) and without (-C) collagenase treatment. Proteins were resolved by NaDODSO₄-5% polyacrylamide gel electrophoresis. Confluent cultures were labeled for 24 h with [¹⁴C]proline and [¹⁴C]glycine in the presence of ascorbate and BAPN as described under Methods and Materials. Duplicate aliquots of media, processed with inhibitors, and cell layer, extracted for 16 h at 4 °C with 0.5 N acetic acid, were desalted by dialysis and lyophilized. Samples were incubated with and without purified bacterial collagenase for 16 h at 35 °C. Lane 1 (S) contains [¹⁴C]proline-labeled chicken calvaria collagen type I. All samples were reduced with DTT.

Aliquots of media were dialyzed against 1% acetic acid, lyophilized, and put on 5% acrylamide gels with and without collagenase. The fluorograms of the gels are also shown in Figure 1. The K16 media contained collagenous $\alpha 1$ and $\alpha 2$ bands as well as procollagen chains. The W8 media had no α -size chains, just a single pro α chain. In a similar experiment, media containing inhibitors were desaluted over a P-2 column and immediately frozen and lyophilized. Aliquots with and without pepsin treatment and/or reduction with DTT were placed on 7.5% polyacrylamide gels as seen in Figure 2. The K16 media still contained $\alpha 1$ - and $\alpha 2$ -size chains as well as procollagen, suggesting that procollagen to collagen conversion occurred in the media during the 24-h pulse period. The W8 media had only procollagen α chains. Therefore, no processing of procollagen occurred in the media of the W8 cell line. After pepsin treatment K16 media contained both $\alpha 1$ and $\alpha 2$ chains. However, W8 had no $\alpha 2$ chains and only an $\alpha 1$ chain. There was no difference between pepsin-treated reduced and pepsin-treated unreduced samples. Therefore, only reduced samples are shown in Figure 2.

Pepsin-Treated Media Proteins. Pepsin-treated K16 and W8 media were fractionated as described under Methods and Materials. The majority of the collagenous proteins in the K16 media were precipitated at 2.6 M NaCl and not at 4.4 M NaCl. Figure 3 shows the α -chain separations on CM-cellulose columns for both precipitations. The K16 2.6 M precipitate had $\alpha 1$ and $\alpha 2$ chains with a 2.5:1 ratio calculated by pooling the remainder of each fraction (4.9 mL) and counting the radioactivity for each α chain. The K16 4.4 M precipitate contained a small amount of $\alpha 1$ chain. On the other hand,

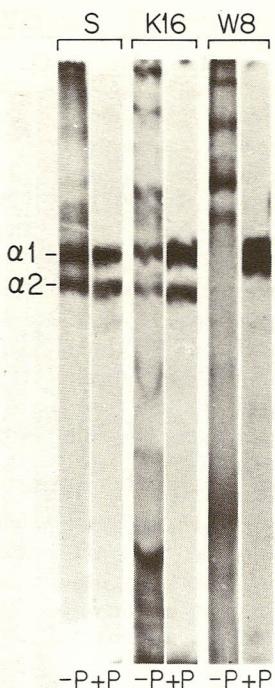


FIGURE 2: Autoradiograms of [¹⁴C]proline- and -glycine-labeled proteins in K16 and W8 media with (+P) and without (-P) pepsin treatment. All samples were reduced with DTT. Proteins were resolved by NaDodSO₄-7.5% polyacrylamide gel electrophoresis. Confluent cultures were labeled for 24 h as described for Figure 1. Duplicate aliquots of media were processed with inhibitors, desaluted by P-2 columns, and then incubated at 4 °C for 16 h with or without pepsin (1:100 dilution). S refers to a standard chicken calvaria type I collagen preparation labeled with [¹⁴C]proline.

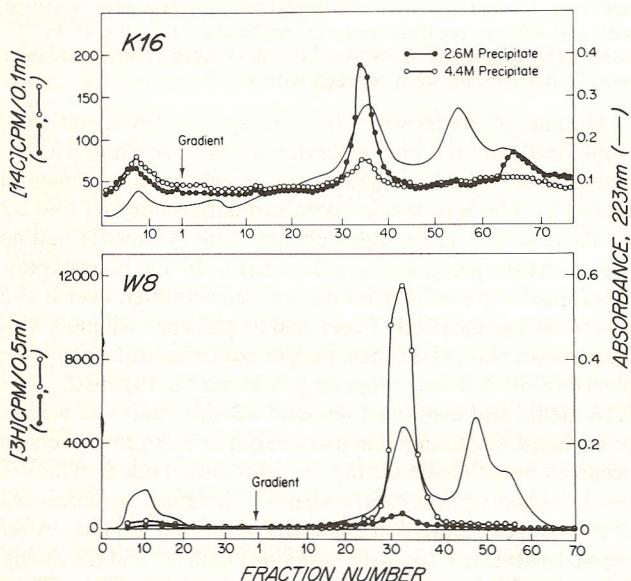


FIGURE 3: CM-cellulose elution pattern of radioactive proline- and glycine-labeled K16 (top) and W8 (bottom) culture medium collagen after pepsin treatment and precipitation with 2.6 M NaCl (●) and 4.4 M NaCl (○). Carrier (type I) collagen (solid line) was isolated from rat skin.

the W8 media had most of their collagenous counts in the 4.4 M NaCl and 0.05 M Tris, pH 7.6, precipitate. The CM-cellulose chromatography of the W8 precipitates (Figure 3, bottom) shows the α-chain composition of the pepsin-treated W8 media. No α2 chains were observed. The main component in W8 pepsin-treated media was an α1 chain precipitated by high salt concentrations. This major peak from CM-cellulose columns contained a single chain migrating on Na-

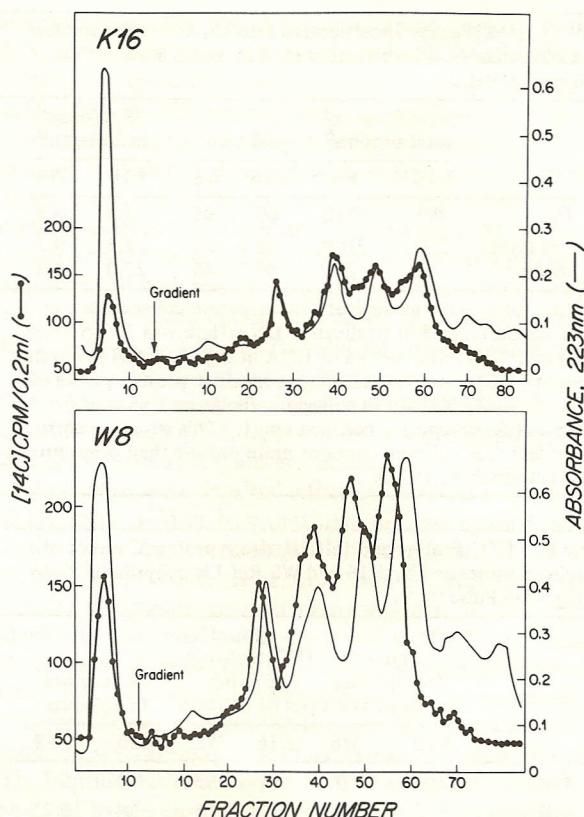


FIGURE 4: CM-cellulose chromatography of cyanogen bromide peptides from [¹⁴C]proline-labeled K16 α1 (top) or W8 α1 (bottom) chains. Collagen chains were isolated from CM-cellulose chromatography (Figure 3) and digested by cyanogen bromide in the presence of α1 and α2 chains (solid line) from rat skin collagen.

DodSO₄ gels in the α1 position.

Cyanogen Bromide Cleavage of α1 Chains. The α1 chains isolated from CM-cellulose columns were digested with cyanogen bromide and examined by CM-cellulose columns. The K16 collagen had characteristic α1-derived peptides (Figure 4, top). The W8 α1 chains had peptides that eluted on CM-cellulose columns slightly ahead of the rat skin α1 cyanogen bromide peptides used as an internal standard (Figure 4, bottom). Each of the peptides isolated from CM-cellulose chromatography was placed on 10% polyacrylamide gels. The fluorograms of the peptides coincided with the standard peptides from rat skin α1 chains (not shown).

Pepsin-Treated Cell Layer Proteins. Pepsin-resistant cell layer proteins were salt fractionated in a similar manner to the media protein. Most of the collagenous molecules in K16 cell layers contained α1 and α2 chains as judged by CM-cellulose chromatography and NaDodSO₄-polyacrylamide gel electrophoresis. As with the media proteins, the majority of the W8 cell layer molecules needed higher salt concentrations. The CM-cellulose chromatography of the W8 cell layer 2.6 M precipitate showed a small peak which eluted after the α1 rat skin collagen standard (not shown). The 4.4 M precipitate contained one broad peak on CM-cellulose chromatography in the α1 region (not shown) which, on 5% polyacrylamide gel electrophoresis, contained three proteins with similar migration to that shown in Figure 1 for the entire W8 cell layer.

Discussion

This paper shows that a rat liver epithelial, cloned cell line, K16, produced a large amount of collagen (Tables I and II) as judged by both hydroxyproline determination and collagenase

assays. A comparison of these data to Green and co-workers' (Green & Goldberg, 1965; Green et al., 1966) original comparisons of hydroxyproline content in a wide variety of cell lines shows that the K16 cells produce as much collagen as many fibroblasts in culture. Others have also shown that rat liver epithelial cells do produce collagen (Sakakibara et al., 1977, 1978; Hata et al., 1979). We have also shown that this cell line produces primarily type I collagen as judged by (1) its salt precipitation in 2.6 M NaCl and 0.05 M Tris, pH 7.6, (2) its chain composition on CM-cellulose chromatography and gel electrophoresis, and (3) the peptide maps of the cyanogen bromide digested peptides. In a recent paper, Hata et al. (1979) partially characterized the collagens produced by several epithelial cell lines. These had variable amounts of type III collagen. The cell line used in this paper produced insignificant amounts of type III collagen.

As indicated in the introduction, previous studies show that transformed fibroblasts synthesize less collagen. The chemically transformed epithelial cells, W8, used in this study maintain their ability to produce collagen. The amount of [¹⁴C]proline incorporated into collagen by the transformed W8 cells at confluence during a 6-h pulse was equal to or slightly greater than the amount incorporated by the K16 parent cells. This was apparent when collagenase assay data and hydroxyproline data were calculated on a per microgram of DNA or cell basis (not shown). On the other hand, the W8 cells incorporated much more proline into noncollagenous and total protein than the K16 cells. Therefore, the relative percent of collagen synthesized by the W8 cells was markedly decreased compared to the K16 cells. The pool size of proline was not measured in this study. It is possible that, due to an increased transport of proline, the specific activity of proline in the cell was higher in the transformant. However, the total protein per cell as measured by the Lowry method and the dry weight of the cell layer were greater for the W8 cells than for the K16 cells, suggesting that the cells did indeed make more protein during the culture period. The large increase in proline incorporation into W8 cells is probably due to a combination of pool size differences as well as increased protein synthesis. As opposed to fibroblast studies, the amount of proline incorporated into collagen by the W8 cells in a 6-h pulse is not lower than the parent cell line. This could reflect the mode of transformation or the different cell type used in these studies.

Hata & Peterkofsky (1977) have shown that the type of collagen produced by 3T3 cells changes after viral and chemical transformation. In this study, transformed W8 cells also displayed altered types of collagen. The cells secreted a procollagen molecule into the media which had only one type of $\alpha 1$ chain as judged by gel electrophoresis or CM-cellulose chromatography. No $\alpha 2$ chain could be demonstrated. The cyanogen bromide digest pattern of the isolated α chain on CM-cellulose chromatography closely resembled the carrier $\alpha 1(I)$ chain. However, the radioactivity eluted ahead of the internal standard peptides. Presumably, this would occur if the W8 $\alpha 1$ peptides were more acidic than the normal peptides. This could possibly be due to less amide groups on glutamic or aspartic acids, to phosphorylation, to additional carboxyl groups on glutamic acid, or, less likely, to over hydroxylation of the lysine or glycosylation of the peptides. Crouch & Bornstein (1978) have found that an all $\alpha 1$ type I collagen produced by amniotic fluid cells contains more hydroxylysine. Human skin (Uitto, 1979) also contains some overhydroxylated $\alpha 1(I)$ chains. In these cases, the cyanogen bromide peptides coeluted with standards on CM-cellulose but did not comigrate

on gel electrophoresis. This is not the case with the W8 $\alpha 1$ cyanogen bromide peptides which eluted earlier than standards on CM-cellulose but migrated with standards on gel electrophoresis. Based on this limited evidence, it is felt that W8 α chains are a more acidic type I α chain containing normal hydroxylation. Several investigators (Mayne et al., 1975; Moro & Smith, 1977; Little et al., 1977; Narayanan & Page, 1976; Benya et al., 1977; Munksgaard et al., 1978; Daniel, 1978) have demonstrated an $[\alpha 1(I)]_3$ molecule which has been termed $\alpha 1(I)$ trimer. Originally, this molecule was postulated by Mayne et al. (1975, 1976) to result from altered gene expression of chondrocytes due to embryo extract, bromodeoxyuridine, or in vitro cellular aging. Subsequently, this type of collagen has been described in association with inflammatory diseases (Narayanan & Page, 1976), solid tumors (Moro & Smith, 1977), the teratocarcinoma cell line (Little et al., 1977), amniotic fluid cells (Crouch & Bornstein, 1978), normal developing tissues in organ cultures (Jimenez et al., 1977; Munksgaard et al., 1978), and human skin (Uitto, 1979). Little et al. (1977) have suggested that the trimer collagen represents an embryonic collagen type. The recent finding (Crouch & Bornstein, 1978) that fetal embryonic cells from amniotic fluid produce varying amounts of trimer molecules indicates that this molecule may reflect differences in differentiation or in the case of tumor cells it may be an indication of dedifferentiation as suggested by Benya et al. (1977). One cannot generalize that all transformed cells dedifferentiate by producing excess $\alpha 1$, since Hata & Peterkofsky (1977) found that the parent 3T3 cells have excess $\alpha 1$ chains whereas the transformed cells do not. It has been suggested that pro $\alpha 1(I)$ trimer is resistant to procollagen processing to collagen (Crouch & Bornstein, 1978). This could be the reason we have found very little processing of W8 media procollagen. Alternatively, there may be reduced amounts of procollagen peptidase activity as demonstrated by Sundarrajan & Church (1978) or Arbogast et al. (1977) for virus-transformed fibroblasts.

Three collagenous protein chains can be extracted from the cell layers of the W8 cells. The chains can be demonstrated by gel electrophoresis, but they did not separate sufficiently on CM-cellulose chromatography for complete characterization. The collagenous protein that migrates slower than the $\alpha 1(I)$ on polyacrylamide gel electrophoresis chromatography could be similar to A and B chains (Burgeson et al., 1976; Chung et al., 1976), C chains (Brown et al., 1978), or basement membrane chains recently described by several authors (Kresina & Miller, 1979; Sage et al., 1979; Timpl et al., 1979). Their elution on CM-cellulose is closer to $\alpha 1(I)$ than that found for A and B chains (Burgeson et al., 1976; Chung et al., 1976) or C chains (Brown et al., 1978). This could reflect a species difference (rat type III and $\alpha 2$ elute differently than human collagens on CM-cellulose chromatography) or could indicate that this is a previously undescribed chain. The collagenous protein migrating on gel electrophoresis between $\alpha 1$ and $\alpha 2$ could also be a previously undescribed α chain, a degradation of basement membrane, or an altered $\alpha 2$ procollagen chain. Since this protein migrates like pN $\alpha 2$ (Smith et al., 1977), possibly the N-terminal portion is altered and therefore was not cleaved from the pro $\alpha 2$ chain by pepsin. If the N terminal is altered, it may not form a triple helix or be secreted. The full characterization of these components will have to await further experimentation to separate the chains.

Recent advances in the molecular biology of collagen have made it possible to isolate chicken collagen cDNA (Frischauf et al., 1978; Sobel et al., 1978) and clone some fragments in

Escherichia coli (Sobel et al., 1978; Lehrach et al., 1979). It should be possible in the future, with these probes, to determine the exact mechanism whereby the collagen synthesis has been altered by the chemical in this cell line. At present, several possibilities exist as to why the transformed cell does not secrete α_2 . (1) The α_2 may be synthesized but not incorporated into a triple helix and secreted. (2) The α_2 may be defective in some way so it is degraded within the cell. This may indeed be the case in view of the recently reported intracellular degradation of collagen (Bienkowski et al., 1978). (3) The messenger RNA for α_2 may be present but not translatable. (4) There may be no messenger RNA for α_2 in these cells. Future studies with these cell lines may give insight into collagen regulation and effects of chemical transformation on cellular functions.

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