Biochimica et Biophysica Acta, 628 (1980) 388—397

BBA 29193

SYNTHESIS OF HYALURONIC ACID AND COLLAGEN IN SKIN FIBROBLASTS CULTURED FROM PATIENTS WITH OSTEOGENESIS IMPERFECTA

HILKKA TURAKAINEN, HANNU LARJAVA, HEIKKI SAARNI and RISTO PENTTINEN

Department of Medical Chemistry, University of Turku, Kiinamyllynk. 10, SF-20520 Turku 52 (Finland)

(Received May 29th, 1979)
(Revised manuscript received November 1st, 1979)

Key words: Hyaluronic acid synthesis; Collagen synthesis; Osteogenesis imperfecta; (Human skin fibroblast)

Summary

Collagen and hyaluronic acid syntheses were studied in skin fibroblast cultures from patients with osteogenesis imperfecta and age-matched controls by labeling the cultures either with [^{3}H]proline and separating the collagenous proteins with DEAE- and CM-cellulose chromatographies, or double-labeling the cultures with [^{3}H]glucosamine and [^{14}C]glycine and separating radioactive hyaluronic acid from glycoproteins and sulphated proteoglycans by DEAE-cellulose chromatography. The activities of the cell layer hyaluronate synthesizing enzyme complex (hyaluronate synthetase) were also determined.

The osteogenesis imperfecta cultures were classified into three variants on the basis of type III collagen synthesis. Type III collagen amounted to approx. 40—50% from total collagen in the first variety and approx. 25—30% in the second variety. No difference was noted in the ratio of type III collagen to total collagen in the third variety in comparison with control cultures.

The radioactivities of ^{3}H-labeled hyaluronic acid in DEAE-cellulose chromatograms were compared with those of the ^{14}C-labeled proteins. The ratios ranged 9.2—17.3 in the cultures from the patients and 4.6—8.8 in the control cultures. Hyaluronate synthetase activities were 1.3—2.0-fold higher in the osteogenesis imperfecta cells than in their controls.

Increased hyaluronic acid synthesis in skin fibroblasts correlated with the severity of the disease but not with the increase in type III collagen synthesis.

Introduction

Osteogenesis imperfecta is a heritable disorder of connective tissue which principally affects skeleton [1] and leads to dislocated tendons, blue sclerae, scoliosis, and loss of hearing. The syndrome can be divided into several variants based on the onset of the symptoms [1,2], on skeletal findings [1], on the colour of the sclerae [2,3] or on the pattern of inheritance [1,4,5].

Previous results on collagen synthesis in cultured skin fibroblasts have shown that osteogenesis imperfecta is biochemically heterogenous [6]. In one lethal form an impaired synthesis of type I procollagen was reflected by a significant increase in the proportion of type III procollagen [6]. In some other cases the increase in type III collagen synthesis was less marked [6,7]. Increased amounts of type III collagen have later been demonstrated in skin [8,9] and bone [10] of certain patients with osteogenesis imperfecta. Type I collagen is the main protein of bone [11] which, in contrast to skin or some other tissues, does not contain type III collagen [10]. However, a number of fibroblast strains from patients did not express any alteration in the synthesis of collagen types [6,12]. High ratio of type III collagen to type I collagen synthesis observed in certain cultures does not, thus, correlate with the clinical manifestation of the disease and its role in the genesis of the disease is not yet clear.

Tissue specimens of patients with osteogenesis imperfecta also show variation in the glycosaminoglycans. An increase in their amounts of woven bone and cartilage [13,14] and in their sulphation [15] have been reported in congenital osteogenesis imperfecta. To the contrary, low chondroitin sulphate content has been recorded in some bone and cartilage specimens [16,17]. The urinary excretion of glycosaminoglycans has been normal in some patients [18].

In the present study hyaluronic acid and collagen syntheses were compared in skin fibroblast cultures from patients with osteogenesis imperfecta and control persons. The results indicate a relative increase in hyaluronic acid synthesis in all osteogenesis imperfecta cases studied.

Materials and Methods

Cells. Fibroblasts were cultured from forearm skin biopsies of patients and age-matched controls (Table I) in Dulbecco's modification of Eagle's medium supplemented with 10% foetal calf serum (both from Flow Laboratories, Irwine, U.K.), 23 mM sodium bicarbonate, 20 mM Hepes (Gibco-Biocult Ltd., Paisley, U.K.), 100 units/ml G-penicillin and 50 μg/ml streptomycin sulphate (Orion Oy Ltd., Helsinki, Finland). Trypsinized cells were stored in liquid nitrogen.

Chromatography of [^{3}H]proline labeled components. All isotopes used for labeling were from the Radiochemical Centre, Amersham, U.K. The medium of confluent cultures was replaced with 5.0 ml Dulbecco's medium, lacking glutamine and serum but supplemented with sodium ascorbate (50 μg/ml), and β-aminopropionitrile (50 μg/ml) as fumarate (both from Sigma Co., St. Louis, MO, U.S.A.) ('labeling medium'). The cultures were exposed for 24 h to 100 μCi [^{3}H]proline (L-[G-^{3}H]proline, spec. act. 630 Ci/mol) in 81 cm^2 cell culture flasks (Nunc Co., Roskilde, Denmark) and cooled on crushed ice. Phenylmeth-

TABLE I

SOURCE OF CELLS

OSTE, ATCC strain No. 1262; BAPOT, ATCC strain No. 1280; other strains cultured from biopsies of local patients. Cells between the 5th and 12th passages were used. NB, newborn; F, female; M, male; OI, osteogenesis imperfacta; C, control; ATCC, American Type Culture Collection, Rockville, MD, U.S.A.

Strain	Age	Sex	Diagnosis
OSTE	NB	F	OI congenita (lethal)
MATI	NB	F	OI congenita (lethal)
KALJU	21 months	M	OI congenita milder course
BAPOT	14 years	M	OI congenita milder course
MARNI	25 years	F	OI tarda
HF	fetal, 20th week of gestation		C legal abortion
TYT	NB	F	C normal skin
LAU	12 years	F	C normal skin
ERTU	18 years	F	C normal skin
KAI	40 years	F	C normal skin

ylsulphonylfluoride (Calbiochem, San Diego, U.S.A., 400 μg in 100 μl 2-propanol) and *p*-chloromercurobenzoate (Fluka AG, Switzerland, 400 μg in 100 μl distilled water) were added to prevent proteolytic alterations of the labeled components.

The media were separated, centrifuged free from cells and dialyzed against 50 mM Na_2EDTA at 4°C for 72 h and against distilled water for 24 h with frequent changes. Cells were detached with a rubber policeman and treated in an analogous way. An aliquot of medium containing 200 000—300 000 cpm was used for the characterization of procollagens with DEAE-cellulose chromatography in 50 mM Tris-HCl buffer, pH 7.4 and in 2 M urea using a linear gradient 0—0.20 M NaCl over a total volume of 400 ml [6,19]. Fractions of 7 ml were collected. An aliquot of medium or homogenized whole culture containing 200 000—300 000 cpm was added to 5 ml of 0.5 M acetic acid solution containing 10 mg of lathyritic rat skin carrier collagen [6], treated with 1 mg pepsin (2696 U/mg, Worthington, NJ, U.S.A.) at 4°C and pH 2, for 4 h [6,19] and neutralized with NaOH at 4°C to inactivate pepsin. The pepsin-resistant collagen chains were separated with CM-cellulose chromatography in 30 mM sodium acetate buffer, pH 4.8 and in 4 M urea at 42°C using a linear gradient 0—0.11 M NaCl over a total volume of 600 ml [6,19]. Fractions of 12 ml were collected.

Chromatography of [^{14}C]glycine and [^{3}H]glucosamine labeled components. For double-labeling experiments confluent cultures in 81 cm^2 flasks were exposed to 50 μCi [^{3}H]glucosamine and 2.5 μCi [^{14}C]glycine, (D-[1-^{3}H]glucosamine hydrochloride, spec. act. 3.2 Ci/mmol; [^{14}C]glycine, spec. act. 107 Ci/mol) in 10 ml of the labeling medium (see above) for 24 h. Protease inhibitors were added and media and cells were separated and dialyzed as described above. The whole media were chromatographed at 4°C in 50 mM Tris-HCl buffer, pH 7.4, containing 2 M urea on DEAE-cellulose using a linear gradient 0—0.60 M NaCl over a total volume of 600 ml. Fractions of 8 ml were collected.

Chromatography of [^{35}S]sulphate labeled components. Confluent cultures were exposed to 5 μCi [^{35}S]sulphate (carrier-free) for 24 h in the labeling medium. The media were dialyzed against ice-cold 0.1 M Na_2SO_4 for 72 h and against 50 mM Tris-HCl buffer, pH 7.4, for 24 h and chromatographed on DEAE-cellulose as in the double-labeling experiments.

Treatment of DEAE-cellulose fractions with collagenase and hyaluronidase. Collagenous proteins were localized in the DEAE-cellulose chromatograms using bacterial collagenase. The fractions were dialyzed, freeze-dried, dissolved into 1.0 ml 0.15 M NaCl solution and digested in 0.1 ml portions with collagenase, (purified from type I; Sigma Co., St. Louis, MO, U.S.A.) [20,21], approx. 5 μg/ml. Undigested radioactivity was precipitated with 6 M perchloric acid and centrifugation at 2000 $\times g$ for 30 min at 4°C. The radioactivities of the supernatants (1 ml) were counted in 10 ml Tergitol scintillation fluid. For localization of hyaluronic acid 0.1 ml samples of the dissolved fractions (1 ml) were treated with testicular hyaluronidase (1 g/l, Sigma Co.) for 6 h at 37°C. The undigested radioactivity was precipitated by adding ethanol to 83% (v/v) and centrifuging at 2000 $\times g$ for 30 min. The radioactivities of the supernatants (1.5 ml) were counted in 10 ml Tergitol scintillation fluid. Some media samples were digested with streptomyces hyaluronidase (Calbiochem, San Diego, U.S.A.; 10 TRU */5 ml medium) before chromatography on DEAE-cellulose.

Assay of the activity of hyaluronate synthesizing enzyme complex (hyaluronate synthetase). Fresh medium was changed to confluent cultures and the cells were allowed to grow for 24 h. They were then washed free from serum with ice-cold 0.9% NaCl solution, collected with a rubber policeman and homogenized by a Pasteur pipette into 500 μl 0.9% NaCl solution. Protein concentration was determined [22], the suspension rapidly frozen, and kept at −70°C until the enzyme assays. A modification of the method described by Ishimoto et al. [23,24] was used. The reaction mixture (30 μl) contained 1.0 μl 1 M Tris-HCl buffer, pH 7.3/1.0 μl 1.5 M $MgCl_2$/2.0 μl 10 mM UDP-*N*-acetylglucosamine/1 μl 0.078 mM UDP-[^{14}C]glucuronic acid (UDP-[DU-^{14}C]glucuronic acid, spec. act. 321 μCi/mol, as ammonium salt)/25 μl of cell homogenate (approx. 100 μg protein/30 μl). Control vials containing the complete mixture were boiled for 5 min. The samples and their controls were then incubated at 37°C for 60 min and frozen. Hyaluronic acid-like polymer was separated from sulphated glycosaminoglycans and its radioactivity was determined as described by Saarni and Tammi [25]. The activity of the enzyme complex was expressed as μmol of UDP[^{14}C]glucuronic acid incorporated into the polymer per min per mg of cell layer protein.

Determination of radioactivity. The radioactivities in 1.0 ml fraction of the chromatograms were counted in 10 ml Tergitol scintillation fluid [21,25] with Packard Tri Carb 3375 or 3320 liquid scintillation spectrometers. The radioactivities of ^{3}H- and ^{14}C-labeled components in double-labeling experiments were counted in separate channels and the overlapping of ^{14}C-radioactivity in the ^{3}H-channel was corrected by a computer. In hyaluronate synthetase assays 2.0 ml samples were counted with 15 ml Tergitol scintillation fluid. The effi-

* TRU, turbidity reducing units.

ciencies of the scintillation fluid were 12—13% for [^{3}H]proline and [^{3}H]glucosamine, 73—80% for [^{14}C]glycine or [^{14}C]glucuronic acid and approx. 90% for [^{35}S]sulphate [21,25].

Results

Classification of the cultures on the basis of type III collagen synthesis

Ion exchange cellulose chromatograms of osteogenesis imperfecta fibroblast culture media (Table I) indicated cell strain specific differences in the ratio of type III to type I collagenous proteins. Fig. 1 shows DEAE-cellulose chromatograms of [^{3}H]proline labeled media procollagens of three variants of osteogenesis imperfecta strains and a control strain. Fig. 2 shows the CM-cellulose chromatograms of media proteins after limited pepsin digestion. The first variant, a well characterized lethal case of congenital osteogenesis imperfecta (ATCC strain No 1262), shows a relative increase in the procollagen type III content (Fig. 1A). Increase in type III collagen (up to 40—50% of total collagens) is obvious also in the CM-cellulose chromatographic pattern (Fig. 2A). Figures 1B and 2B show respective chromatograms of the second variant, a case of osteogenesis imperfecta tarda. CM-cellulose chromatogram of pepsin treated media proteins shows that type III collagen consists approx. 25—30% of total collagens (Fig. 2B). Chromatograms of some other strains, e.g. ATCC strain No 1280, (congenital cases with a milder course) show also similar patterns.

Respective media chromatograms of the third variant are shown in Figs. 1C and 2C. The cells were cultured from another lethal case. Repeated labelings and chromatograms failed to show any increase in type III collagen synthesis compared with the controls (Figs. 1D and 2D) and are similar to chromatograms of a number of other osteogenesis imperfecta congenita or tarda

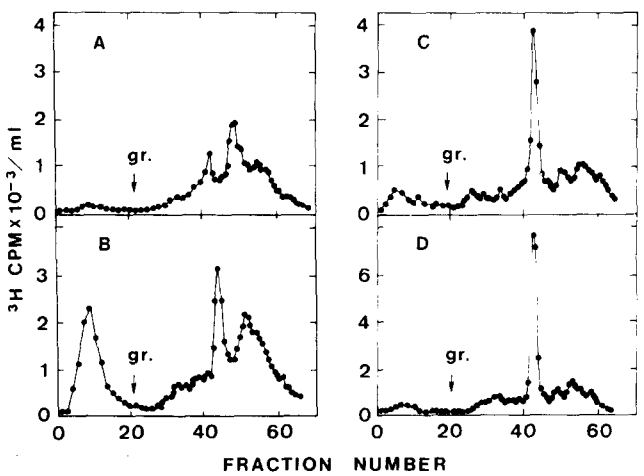

Fig. 1. DEAE-cellulose chromatograms of [^{3}H]proline labeled media proteins of three osteogenesis imperfecta skin fibroblast strains and one control strain. The chromatograms were run at 4°C. Procollagen type I is eluted as a sharp peak in fractions 41—47. Material eluting in fractions 49—56 contains procollagen type III. The start of the gradient (gr.) is indicated with an arrow.

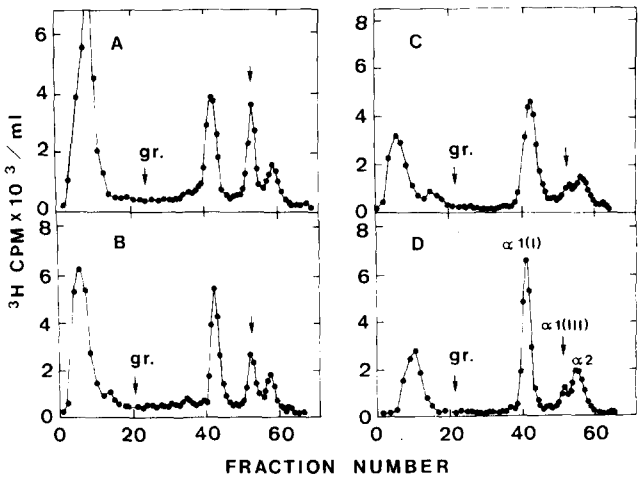

Fig. 2. CM-cellulose chromatograms of [^{3}H]proline labeled media proteins of three osteogenesis imperfecta strains and one control strain. Prior to chromatography the procollagens were converted to collagen size molecules with limited pepsin treatment. The chromatographies were run at 42°C. The start of the gradient (gr.) is indicated with an arrow. The positions of type I collagen α1 and α2 chains and type III collagen [α1(III)]$_3$ are indicated in the control chromatogram. Type III collagen peaks in other chromatograms are indicated with arrows.

strains, and of a normal fetal skin fibroblast strain. Chromatograms of pepsin treated whole cultures did not differ from those obtained from media proteins.

Separation of radioactive hyaluronic acid from other labeled components with DEAE-cellulose chromatography

The pattern of [^{3}H]glucosamine-[^{14}C]glycine labeled cultured media chromatograms of the two lethal strains are shown with their controls in Fig. 3. Procollagen, hyaluronic acid and sulphated proteoglycans were localized in the fractions with collagenase and bovine and streptomyces hyaluronidase treatments. Variable amounts (36—78% of total radioactivity) of collagenase sensitive material [19,26] were detected in the fractions eluting at 0.09—0.20 M NaCl (marked with 1) whereas the fraction eluting at 0.22—0.29 M NaCl (marked with 2) was almost completely (72—91%) bovine testicular hyaluronidase sensitive. This fraction disappeared completely in chromatograms of samples digested with streptomyces hyaluronidase. Sulphated proteoglycans were eluted at 0.40—0.58 M NaCl concentration.

All three variants of osteogenesis imperfecta fibroblasts showed an increase in the radioactivity of the hyaluronic acid peak compared with their controls (Table II). The synthetic rate of hyaluronic acid (and collagen) were, however, highest in human fetal skin fibroblast cultures. The ratios of ^{3}H-radioactivity of the hyaluronic acid fractions to the ^{14}C-radioactivity of the protein peaks in the chromatograms were higher in all cultures from the patients (range 9.2—17.3) than in their age-matched controls, or in fetal skin fibroblasts (range 4.6—8.8) ($P < 0.005$), (paired t-test, $n = 5$). No difference was detected in the radioactivities of sulphated glycosaminoglycan peaks.

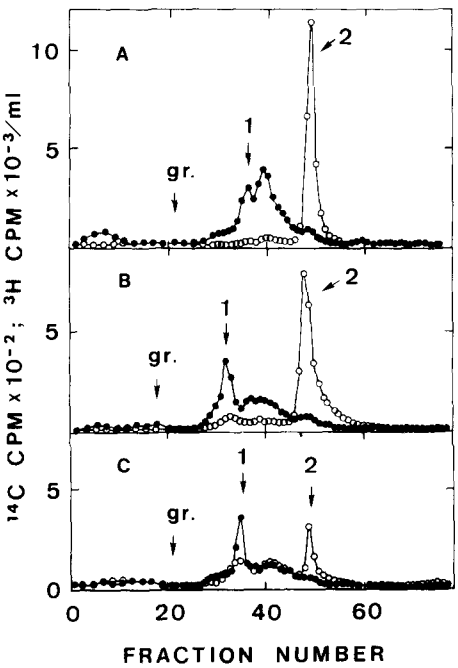

Fig. 3. DEAE-cellulose chromatographic patterns of media components from skin fibroblast cultures of two lethal types of osteogenesis imperfecta and a control double-labeled with [^{3}H]glucosamine and [^{14}C]glycine. The start of the gradient (gr.) and position of procollagen type I (1) and hyaluronic acid (2) are indicated with arrows. A, ATCC strain 1262; B, strain MATI; C, newborn control strain.

The activity of hyaluronate synthetase

The activities of hyaluronate synthetase were higher in the cultures from patients than in the cultures from their age-matched controls (Table III). The highest activities were recorded in cultures from the lethal strains. As expected,

TABLE II

RADIOACTIVITIES OF PROTEINS AND HYALURONIC ACID IN FRACTIONS OF DEAE-CELLULOSE CHROMATOGRAMS FROM OSTEOGENESIS IMPERFECTA AND CONTROL SKIN FIBROBLAST CULTURES

The cultures were double-labeled with [^{3}H]glucosamine and [^{14}C]glycine and the media radioactive components were separated with DEAE-cellulose chromatography. OI, osteogenesis imperfecta; C, control HA, hyaluronic acid.

Strain		HA (^{3}H, cpm)	Protein (^{14}C, cpm)	^{3}H/^{14}C ratio
OSTE	OI	56 964	6225	9.2
MATI	OI	31 445	2586	12.2
KALJU	OI	23 613	2217	10.7
BAPOT	OI	35 177	2482	14.2
MARNI	OI	29 393	1697	17.3
HF	C	59 459	7434	8.0
TYT	C	9 317	2019	4.6
ERTU	C	21 266	2416	8.8
KAI	C	17 150	2451	7.0

TABLE III

THE ACTIVITY OF HYALURONATE SYNTHETASE IN OSTEOGENESIS IMPERFECTA SKIN FIBROBLASTS AND THEIR CONTROLS

The means of the protein concentrations and the activities of the enzyme (per mg cell layer protein and the relative activities) are given (three determinations in each experiment). 25 μl of the total homogenate (500 μl) was taken for the assays. OI, osteogenesis imperfecta; C, control; n.s., = not significant (*t*-test).

Experiment	Strain		mg protein/ assay	pmol/min per mg protein $\times 10^{-2} \pm$ S.E.	Relative activity (range)
1	OSTE	OI	97.0	36.7 ± 0.1 *	1.72—1.73
	MATI	OI	80.0	39.8 ± 2.7 **	1.74—2.00
	TYT	C	84.5	21.3 ± 0.3	1.00
	HF	C	93.0	31.1 ± 5.1 **	1.40—1.53
2	BAPOT	OI	86.5	19.9 ± 0.8 ***	1.27—1.38
	LAU	C	68.5	15.0 ± 1.4	1.00
3	MARNI	OI	73.5	33.6 ± 2.3 n.s.	1.30—1.50
	ERTU	C	55.0	24.0 ± 4.9	1.00

* $P < 0.001$.
** $P < 0.01$.
*** $P < 0.05$.

human fetal skin fibroblasts had more activity than cultures from newborn skin.

Discussion

Analyses of tissue specimens obtained from patients with osteogenesis imperfecta have pointed to disturbed proteoglycan metabolism [13—17]. Variation in histological structure of the specimens is possible and thus more studies are needed.

No previous reports have been published on hyaluronic acid synthesis in osteogenesis imperfecta. A glycopeptide containing galactose and iduronic acid has been characterized from urine of certain patients [27]. Its origin is not known.

Fibroblast cultures exposed to [^{3}H]glucosamine produce radioactive procollagen and other glycoproteins, hyaluronic acid and proteoglycans. [^{3}H]glucosamine label followed to the pattern of [^{14}C]glycine labeled procollagens (Fig. 3) indicating labeling of the procollagen extension peptides. Similar DEAE-cellulose chromatograms have been obtained from procollagens labeled with [^{3}H]glucosamine or [^{3}H]mannose [26]. Compared with the [^{14}C]glycine labeled proteins (Fig. 3, Table II) the radioactivities of [^{3}H]hyaluronic acid peaks were increased in all osteogenesis imperfecta cultures studied. The ratio of radioactivities in [^{3}H]hyaluronic acid/[^{14}C]protein were also higher in media chromatograms of those cultures than the ratios of fetal skin fibroblast cultures which produced hyaluronic acid at a high rate (Table II). These results were confirmed by the activities of the hyaluronate synthetase (Table III). In each particular experiment the activity of the synthetase complex in the patient's cells was higher than in the respective age-matched control culture.

It is possible, however, that nonsulphated precursors of, e.g., heparan sulphate are formed in excess in osteogenesis imperfecta. Such precursors could be eluted together with hyaluronic acid in the DEAE-cellulose chromatography and in the fractionation of Saarni and Tammi [25]. In control fibroblast cultures (in conditions expected to allow normal sulphation) 5—10 times more radioactivity is incorporated into the hyaluronic acid-like fraction than in the sulphated glycosaminoglycans (Fig. 3). Thus major contamination of hyaluronic acid with nonsulphated glycosaminoglycan precursors seems unlikely but such a possibility is currently under investigation.

Hyaluronic acid synthesis is high during the growth of experimental granulation tissue [28] or during proliferation of fibroblasts in culture [29]. Addition of fresh medium induces mitotic activity and activates hyaluronic acid synthesis, probably because of factors present in the serum [30]. Incorporation experiments were therefore carried out without serum in the medium.

Increased synthesis of type III collagen has been detected in cells cultured from many patients with osteogenesis imperfecta [6,7]. Classification of the cell strains into three groups on the basis of relative type III collagen synthesis might be useful for further biochemical research on this syndrome. The highest activity of the hyaluronic acid synthetase (Table III) was observed in cultures of two lethal cases. The first of these strains ATCC 1262, variant I, had also the most severe defect in the metabolism of collagen (Figs. 1A, 2A) whereas the other strain, variant III, had no obvious alterations in the ratio of type III to total collagens estimated with ion exchange cellulose chromatographies. Several other cells strains were classified on the basis of their collagen synthesis. Their majority seems to belong to osteogenesis imperfecta variant II. In addition to the strain in Fig. 1C several other strains, e.g. ATCC strains 1288 and 1267, do not show any alteration in the ratio of type III collagen to total collagen. It is possible that the actual amounts of type I and type III collagens are normal after translation and that type I collagen is degraded during the posttranslational events in osteogenesis imperfecta variants I and II.

Abe et al. [31] noted that fibroblasts in high density produce more type III collagen than those seeded in low density. In the present experiments confluent cultures were used and the results cannot be explained on the basis of small variations in cell densities in such cultures.

Mechanical weakness of connective tissue is a cardinal sign of two common inherited connective tissue disorders, the osteogenesis imperfecta and Marfan's syndromes [1,5]. In the latter the weakness is located in large arteries, whereas skeletal fragility predominates in osteogenesis imperfecta. Increased synthesis of hyaluronic acid has also been noted in fibroblasts cultured from Marfan's syndrome patients [32].

It can be speculated that connective tissue weakness in these syndromes might maintain a continuous activation or 'repair' status of the fibroblasts which is reflected by high type III collagen and hyaluronic acid synthesis. Type III collagen synthesis is also high in early granulation tissue in wound healing [33], and both the concentration of type III collagen and hyaluronic acid [6,28] are higher in embryonic tissues than in respective adult tissues. Histological sections of tissues from patients with osteogenesis imperfecta express various signs of immaturity (see, for example, Ref. 1). We do not yet know if

the fibroblasts of the individuals suffering from osteogenesis imperfecta express high synthesis of hyaluronic acid in vivo or if their hyaluronic acid is abnormal.

Acknowledgements

We thank Dr. Ilkka Kaitila, Dept. of Pediatrics, University of Helsinki, and Dr. Leila Risteli, Dept. of Medical Biochemistry, University of Oulu, for providing us with two osteogenesis imperfecta fibroblast strains. This study is partially supported by a research contract from the Medical Research Council of the Academy of Finland, by grants from The Finnish Cultural Foundation and the Finnish Dental Society (H.L.).

References

1 McKusick, V.A. (1972) Heritable Disorders of Connective Tissue, pp. 390—454, 4th edn., Mosby Co., St. Louis
2 Francis, M.J.O., Smith, R. and Bauze, R.J. (1974) Brit. Med. J. 1, 421—424
3 Francis, M.J.O., Bauze, R.J. and Smith, R. (1976) Birth Defects, Orig. Article Ser. 11, 99—102
4 Sillence, D.O. and Rimoin, D.L. (1978) Lancet 8072, 1041—1042
5 McKusick, V.A. (1978) Mendelian Inheritance in Man., 5th edn., The Johns Hopkins Univ. Press, Baltimore
6 Penttinen, R.P., Lichtenstein, J.R., Martin, G.R. and McKusick, V.A. (1975) Proc. Nat. Acad. Sci. U.S.A. 72, 586—589
7 Müller, P.K., Lemmen, C., Gay, S. and Meigel, W.N. (1975) Eur. J. Biochem. 59, 97—104
8 Sykes, B., Francis, M.O.J. and Smith, R. (1977) N. Engl. J. Med. 296, 1200—1203
9 Fujii, K., Kajiwara, T. and Kurosa, H. (1977) FEBS Lett. 82, 251—254
10 Müller, P.K., Raisch, K., Matzen, K. and Gay, S. (1977) Eur. J. Pediat. 125, 29—37
11 Miller, E.J. (1976) Mol. Cell. Biochem. 13, 165—192
12 Trelstad, R.L., Rubin, D. and Gross, J. (1977) Lab. Invest. 36, 501—508
13 Spencer, A.T. (1962) J. Path. Bact. 83, 423—427
14 Bleckmann, H., Kresse, H., Wollensak, J. and Buddecke, E. (1971) Z. Kinderheilk. 110, 74—84
15 Engfeldt, B. and Hjerpe, A. (1976) Acta Path. Microbiol. Scand. Sect. A 84, 488—494
16 Solheim, K. (1969) J. Oslo City Hosp. 19, 193—199
17 Cetta, C., Lenzi, L., Rizzotti, M., Ruggeri, A., Valli, M. and Boni, M. (1977) Conn. Tissue Res. 5, 51—58
18 Berggren, L., Wessler, E. and Wennerström, J. (1969) Acta Ophthalmol. 47, 122—128
19 Lichtenstein, J.R., Byers, P.H., Smith, B.D. and Martin, G.R. (1975) Biochemistry 14, 1589—1594
20 Peterkofsky, B. and Diegelman, R. (1971) Biochemistry 10, 988—994
21 Vuorio, E. (1977) Scand. J. Clin. Lab. Invest. Suppl. 149
22 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
23 Ishimoto, N., Temin, H.M. and Strominger, J.L. (1966) J. Biol. Chem. 241, 2052—2057
24 Tomida, M., Koyama, H. and Ono, T. (1974) Biochim. Biophys. Acta 338, 352—363
25 Saarni, H. and Tammi, M. (1977) Anal. Biochem. 81, 40—46
26 Clark, C.C., Kefalides, N.A. and Albrecht, M.E. (1978) J. Biol. Chem. 253, 47—51
27 Cetta, G., Balduini, C., Valli, M., Tenni, R., Lenzi, L. and Castellani, A. (1979) Proc. 6th Colloq. Fed. Eur. Conn. Tissue Clubs, Creteil, August 1978, pp. 157—158
28 Lehtonen, A. (1968) Acta Physiol. Scand. Suppl. 310
29 Moscatelli, D. and Rubin, H. (1975) Nature 254, 65—66
30 Kurtz, M.J. and Stidworthy, G.H. (1975) Biochim. Biophys. Acta 399, 90—100
31 Abe, S., Steinmann, B.V., Wahl, L. and Martin, G.R. (1979) Nature 279, 442—444
32 Lamberg, S.J. and Dorfman, A. (1973) J. Clin. Invest. 52, 2428—2433
33 Gay, S., Viljanto, J., Raekallio, J. and Penttinen, R. (1978) Acta Chir. Scand. 144, 205—211