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ADULT HUMAN SKIN MAINTAINED IN ORGAN CULTURE: I. THE ULTRASTRUCTURE OF THE ACELLULAR COMPARTMENT OF CONNECTIVE TISSUE

Bent Hentzer and Takasi Kobayasi

University of Copenhagen, Department of Dermatology (with Connective Tissue Research Laboratories), Rigshospital, Copenhagen, Denmark

Abstract. Collagenolytic enzymes are produced by cultured skin explants. During cultivation the acellular compartment of the dermal connective tissue is digested away. The ultrastructure of the connective tissue resorption has been studied in human skin maintained in organ culture for 2–10 weeks. The connective tissue changes are: (1) Collagen fibrils with normal axial periodicity but decreasing diameters. On cross sections these fibrils have irregular outlines, while the longitudinal sections show fibrils separated into thin bundles of filaments 40–80 Å thick with preserved normal axial periodicity. (2) Cross-banded filamentous aggregations (CBFA) consisting of fine parallel filaments 25 Å thick and without axial periodicity. The CBFA has 350 Å thick bands at 525 Å intervals. At the end of the cultivation, the CBFA are replaced by solitary filaments 25 Å thick. (3) The elastic fibres show no marked changes of the amorphous matrix, while the fibrils disappear. The loss of collagen and elastic fibrils during cultivation of the skin gives direct evidence of connective tissue degradation. The described changes are discussed in relation to previous ultrastructural studies on connective tissue resorption of various tissues.

Key words: Connective tissue resorption; Skin culture

Connective tissue resorption in cultured human skin explants has not attracted much attention, although it has been known for the last 15 years that cultivated tissue fragments do produce collagenase (14). Eisen et al. (7) demonstrated that medium obtained from cultures of human skin explants contained collagenase activity. Three years later, Eisen et al. (10) found the presence *in vivo* of collagenase in human skin. Evidence has therefore been presented in favour of a degradation mechanism in skin. In a histological study, Weissmann & Fell (42) demonstrated that the intercellular collagen fibres of rat dermis were digested away during cultivation. With this knowledge in mind we expected fo find connective tissue resorption in human skin explants maintained in organ culture.

Ultrastructural studies on connective tissue resorption in skin explants have not been reported hitherto, and little is known of the fine structure of human connective tissue breakdown. Previous ultrastructural studies on connective tissue degradation have so far been based on various animal models, i.e. postpartum involution of the uterus (1, 29), tadpole tail (41), carrageenin granuloma (30), and periodontal ligament (39). The aim of this work was to describe the ultrastructural changes of the acellular compartment of connective tissue as related to cultivation periods, while the cellular collagen fibril resorption will appear in a separate paper (18).

MATERIALS AND METHODS

Skin

Split-thickness skin grafts from 20 healthy individuals were obtained from the Department of Plastic Surgery, Rigshospital, Copenhagen (Table I). The skin grafts were cut into 1 to 3 mm squares with a scalpel blade. The total number of explants studied was 384 (Table I).

Culture procedure

The medium was made up of Eagle's minimum essential medium with Earle's salts, and supplemented with 20% heat-inactivated fetal calf serum; 100 IU/ml penicillin,[1] 50 μg/ml streptomycin,[1] and 50 μg/ml gentamycin.[1] The pH was buffered at 7.4 with 1 M Hepes.[1]

The explants were placed with the dermal side down and pressed gently against the steel grid of a Falcon® organ culture dish. Four to eight explants were placed in each dish. Approx. 2 ml of fresh medium was added to each dish, just covering the top of the explants. The medium was changed twice a week. The cultures were placed in a humidified incubator (Ehret®) at 37°C±0.5°C and gassed with 5% CO_2 in atmospheric air. The explants were harvested every day during the first week and every week during the following 2–10 weeks' cultivation period.

[1] GIBCO, Bio-cult. Glasgow. Scotland.

Table I.

Age (y.)	Anatomic region	Cultivation period (days)	Number of explants
Female			
31	Mamma	19	16
26	Mamma	21	16
32	Mamma	21	16
48	Mamma	21	16
18	Mamma	21	16
19	Mamma	21	16
53	Mamma	20	16
43	Mamma	22	16
26	Mamma	42	32
29	Mamma	20	12
28	Mamma	14	8
39	Mamma	14	8
27	Mamma	14	8
46	Mamma	70	80
61	Mamma	14	8
Male			
42	Abdomen	28	12
54	Abdomen	36	20
59	Abdomen	50	32
26	Face	49	24
58	Face	28	12

Uncultivated skin pieces were taken as controls of each individual.

The influence of the cultivation medium on non-viable explants was studied under identical conditions. Prior to cultivation, the control explants were frozen three times at −20°C and subsequently thawed at room temperature.

Bacteriological examination of the medium grown in organ culture dishes during the cultivation period was repeatedly performed. Contaminated cultures were immediately discarded.

Electron microscopy

The explants were fixed in 3% glutaraldehyde in cacodylate buffer (pH=7.4) with 7.5% sucrose for one day and postfixed in 1% osmium tetroxide for one hour. The specimens were dehydrated in an ethanol series of increasing concentration and finally embedded in Epon 812. Sections were cut on Reichert and LKB ultramicrotomes for light and electron microscopy and stained with 0.1% toluidine blue and lead citrate plus uranyl acetate, respectively. Special staining for electron microscopy was performed on selected specimens. These stainings included the periodic acid silver proteinate method for glycoproteins (40) and the ruthenium red method for proteoglycans (25). The specimens were studied with a Siemens electron microscope (Elmiskop IA) with double condensors at 80 kV.

RESULTS

Changes of the acellular compartment of the connective tissue were found in cultivated explants from all individuals. The changes were first noticed at the 3rd day of cultivation, thereafter gradually increasing in relation to the cultivation period. The connective tissue changes were most predominant in the upper part of dermis (Fig. 1*a*). They were not evenly distributed, and frequently appeared focal (Fig. 1*a*). In the deeper part, the changes mostly appeared around cellular structures, i.e. blood vessels, nerves (Fig. 5*a*), and smooth muscles. All explants remained viable as judged by the presence of intact cells throughout the culture period.

1. *Collagen fibrils*

The collagen fibril changes are divided into two groups, A and B. Although both types of collagen fibril changes were seen in the same explants, type A changes were the more predominant in short-term cultivated explants, while type B disintegration was observed more frequently in long-term explants.

Type A change was native collagen fibrils with normal axial periodicity but reduced diameters. At the end of the cultivation period, most of the collagen fibrils disappeared in the superficial part of corium (Fig. 4*a*). During explantation, a complete degradation of collagen fibrils appeared in some areas, whereas, in others, bundles of fibrils were still present (Fig. 1*a*). Both normal and changed fibrils appeared in one and the same bundle. The diameters of collagen fibrils decreased during cultivation towards 150–300 Å from 550–650 Å (Figs. 1*c*, 2*c*). On cross sections, collagen fibrils with decreased diameters frequently appeared with irregular margins (Fig. 2*c*). On longitudinal sections the fibrils showed decreased thicknesses and tapered ends (Fig. 1*b*). At the ends, the collagen fibrils separated into fine bundles or filaments in a brush-like pattern (Fig. 2*b*). This filament separation also involved rather long segments of the native collagen fibril (Fig. 2*a*) on cross sections, appearing as irregular granular figures (Fig. 2*c*).

The thin filaments were arranged in parallel straight bundles with normal axial periodicity (Fig. 2*a*). The individual filament was 40–80 Å thick and showed no distinct periodicity (Fig. 2*a*, *b*).

The *type B change* was a cross-banded filamentous aggregation (CBFA). The location was the same as the above-mentioned type A change. Native collagen fibrils in large areas of dermis were replaced by these banded structures (Fig. 1*a*, 4*a*). Altered native collagen fibrils frequently appeared

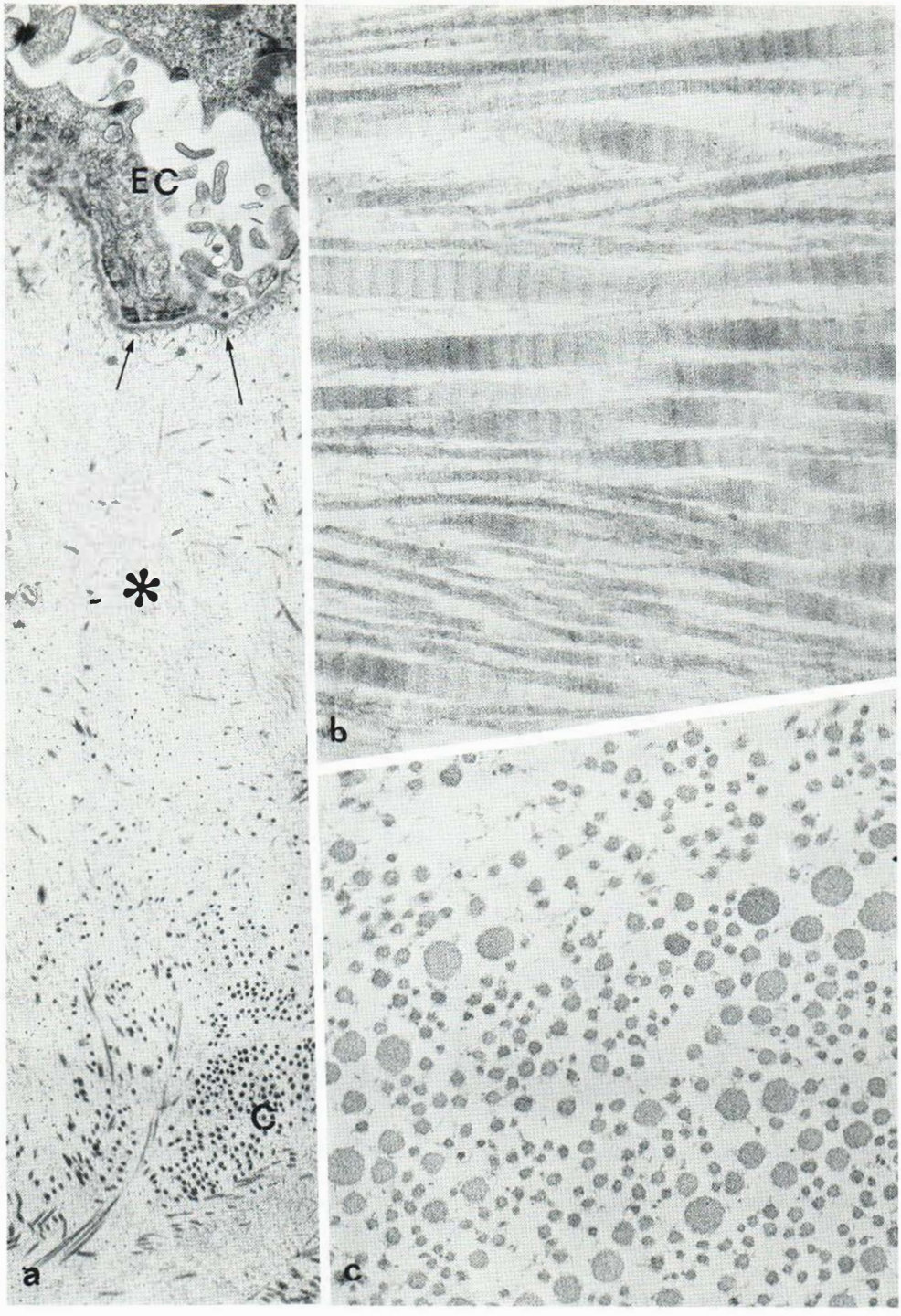

Fig. 1. Skin explant cultivated for 2 weeks. (*a*) The collagen fibrils have disappeared in the upper part of dermis (*asterisk*), while in the deeper part some collagen fibrils (*C*) are still present. The epidermal cells (*EC*) and dermo-epidermal junction (*arrows*) are preserved. ×13950. (*b*) The longitudinal section shows collagen fibrils with varying thicknesses and tapered ends, while the axial periodicity appears normal. ×55800. (*c*) The cross section shows varying diameters of the collagen fibrils. ×55800.

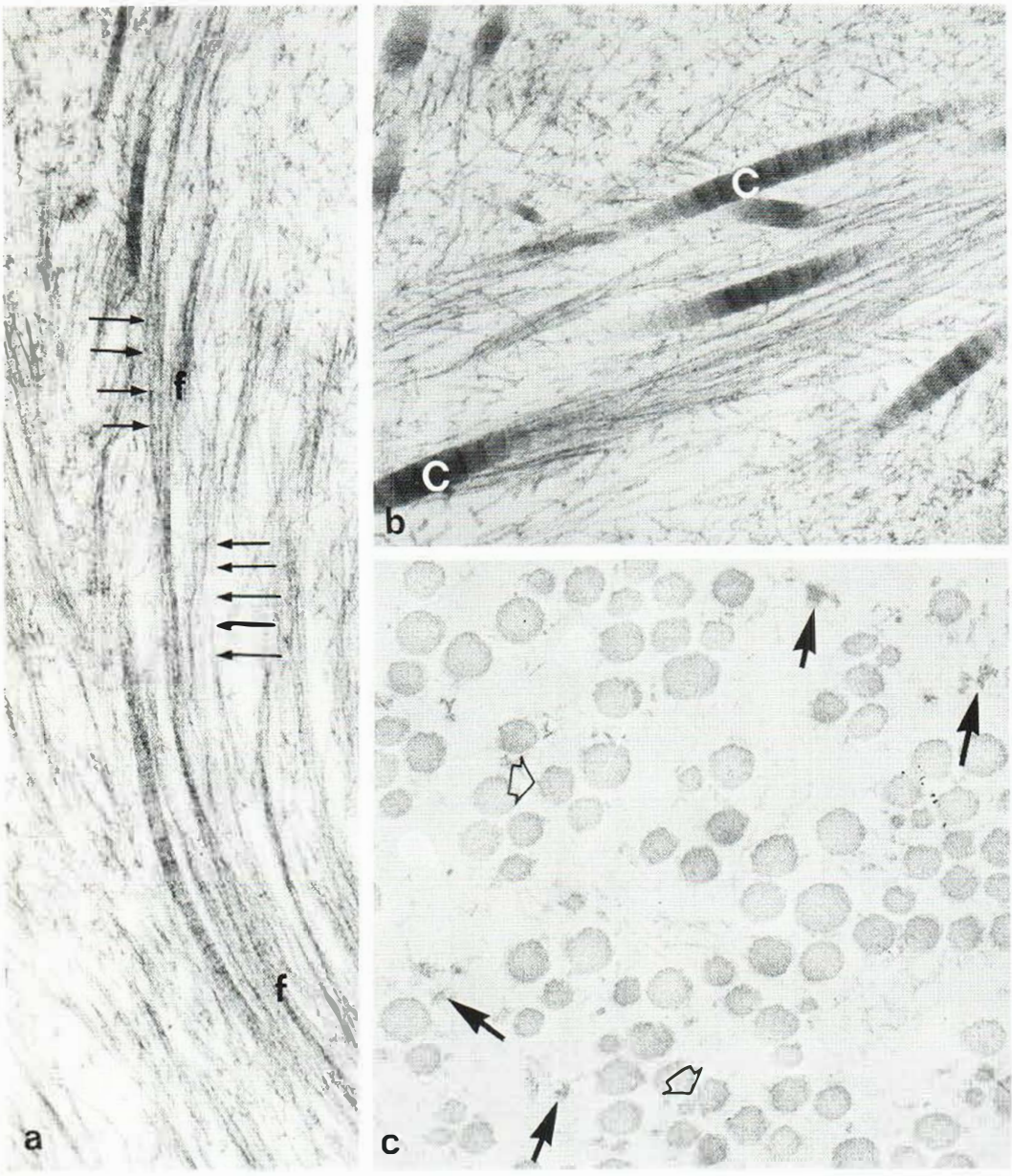

Fig. 2. Collagen fibril changes on the 10th day of cultivation. (*a*) The bundles of filaments (*f*) demonstrate a periodicity similar to that of collagen fibrils (*arrows*). ×60 000. (*b*) A brush-like filament separation is shown at the ends of collagen fibrils (*C*). ×60 000. (*c*) On cross-section, the collagen fibrils appear with irregular margins (*framed arrows*). Filament bundles are seen in the vicinity of collagen fibrils (*arrows*). ×60 000.

in the vicinty of CBFA (Fig. 4*b*). When collagen fibrils were present, the long axis of CBFA was parallel to them (Fig. 4*b*). The ultrastructural morphology of CBFA was very constant and was characterized by fine, mainly parallel filament-bundles with dense cross bands. The individual filament was about 25 Å across (ranging from 15 to 40 Å). On an average, the electron-dense bands were 350 Å thick (ranging from 300 to 425 Å) and consisted of a network of criss-crossing filaments (Fig 4*c*). The width and the length of the CBFA varied considerably. Staining with ruthenium red showed no

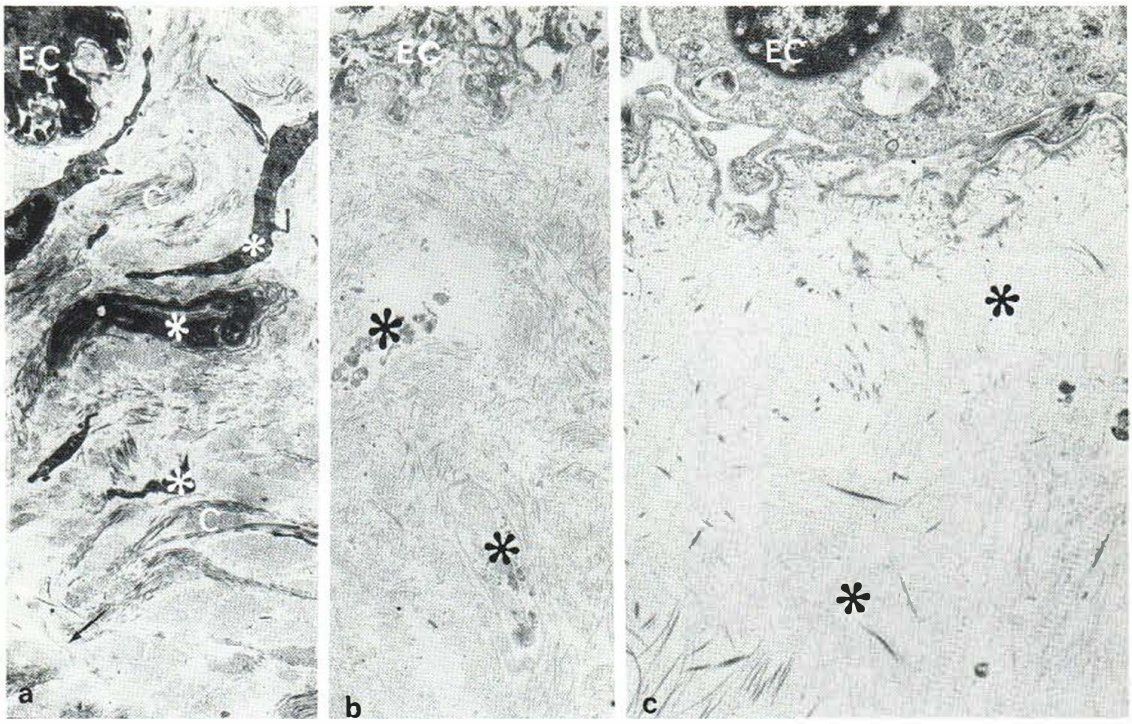

Fig. 3. (*a*) The explant before cultivation (control) shows viable cells of epidermis (*EC*) and dermis (*asterisks*). The dermis is dominated by native collagen fibrils (*C*) and few elastic fibres (*arrow*). ×4 385. (*b*) A previously frozen and thawed explant (control) maintained in serum-enriched culture medium for 3 weeks shows no changes in intercellular connective tissue, while the epidermal cells are totally disintegrated (*EC*). Remnants of cells are present in dermis (*asterisks*). ×4 560. (*c*) Fourteen days of cultivation. In the upper part of dermis, collagen fibrils have disappeared and are replaced by CBFA (*asterisks*). The epidermal cells are viable (*EC*). ×11 400.

increased electron density at the bands, while the periodic acid silver proteinate staining revealed a slight positive reaction.

The CBFA increased significantly during the cultivation period. After 3–10 weeks, there was an increase of solitary filaments, not arranged in bundles, and devoid of periodicity (Fig. 4*d*). These filaments were randomly distributed, but were usually found in the vicinity of CBFA. The solitary filaments had the same diameter as the individual filament of CBFA. Their length could not be determined. After 6 weeks of cultivation, a complete replacement of collagen fibrils by the solitary filaments appeared in the subepidermal part of the dermis (Fig. 4*d*). Between the sixth and the tenth week of cultivation, the thin filaments gradually disappeared, leaving cells and elastic fibres (Fig. 6*a*).

In the vicinity of peripheral nerves of one explant cultivated for 3 weeks, CBFA-like structures appeared with an altered morphology (Fig. 5*a*, *b*, *c*). The periodicity was larger, with an average of 1 250 Å (ranging from 750 to 1 500 Å). The dense bands were 350 Å and the intervals were approximately 920 Å. These structures were always seen in the perineurium and appeared spindle-shaped. The filaments of the spindle tended to fuse in the bands. Frequently, sub-banding of the dense bands was noted (Fig. 5*c*). The individual filament had no periodicity and measured approximately 40–70 Å across (Fig. 5*b*).

II. *Elastic fibres*

The elastic fibres consisted of two components, viz. the amorphous matrix and the fibrils. The amorphous matrix showed no changes during the cultivation. The fibrils, however, both at the periphery and in the interstices of the matrix, decreased in number during the cultivation period (Fig. 6*a*, *b*). No intermediate changes of the morphology or size were

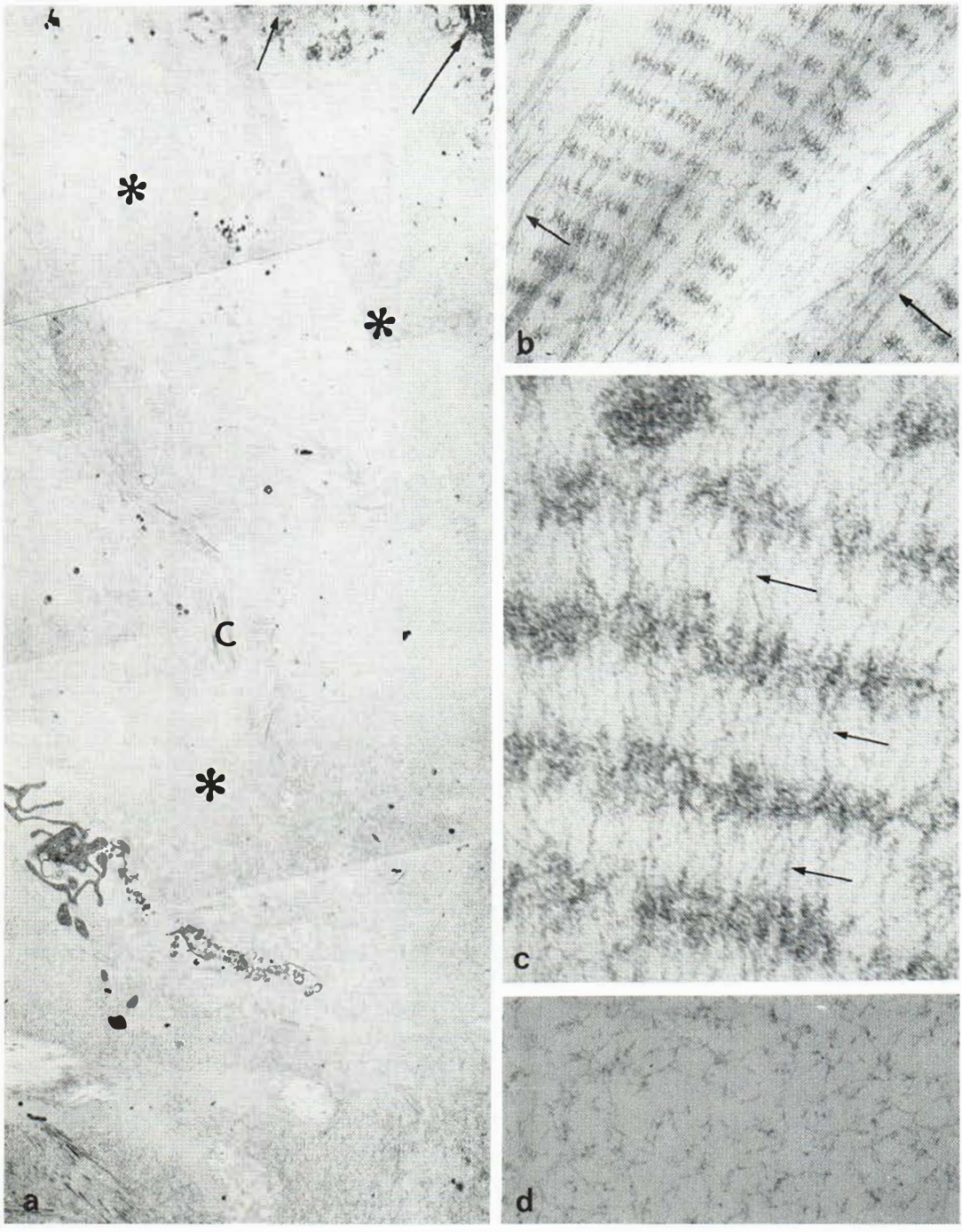

Fig. 4. Connective tissue changes after 3 weeks of cultivation. (*a*) The collagen fibrils are replaced by CBFA (*asterisks*). Larger magnifications are shown in (*b*) and (*c*). The dermo-epidermal junction is preserved (*arrows*). ×6460. (*b*) The long axis of the cross-banded filamentous aggregations is parallel to those of the collagen fibrils, while the periodicity is longer spaced. The filaments as seen in (*a*) are also seen between CBFA (*arrows*). ×57000. (*c*) The spacing of CBFA is 525 Å, while the thickness of the dense bands is 350 Å. The thickness of the filaments (*arrows*) is 25 Å on average. ×228000. (*d*) After the sixth week of cultivation, the solitary filaments of CBFA are randomly distributed, and the CBFA gradually disappear. ×28500.

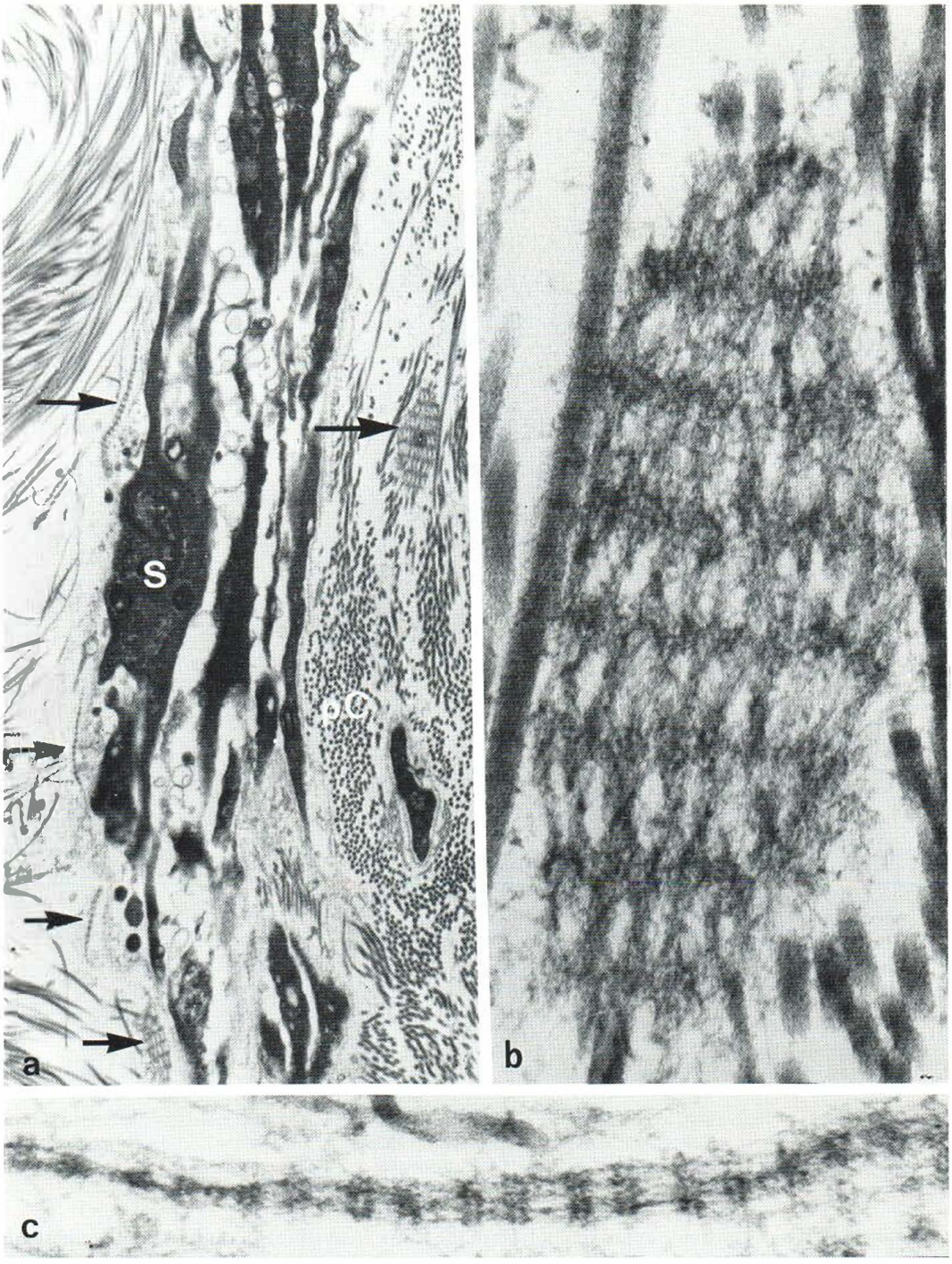

Fig. 5. (*a*) Perineural CBFA-like structures (*arrows*) are occasionally seen in the vicinity of Schwann cells (*S*) mixing with perineural collagen fibrils (*pC*). ×15000. (*b*) The spacing of the spindle-shaped structures is 1250 Å. ×120000. (*c*) Sub-banding of the dense bands is frequently present. ×120000.

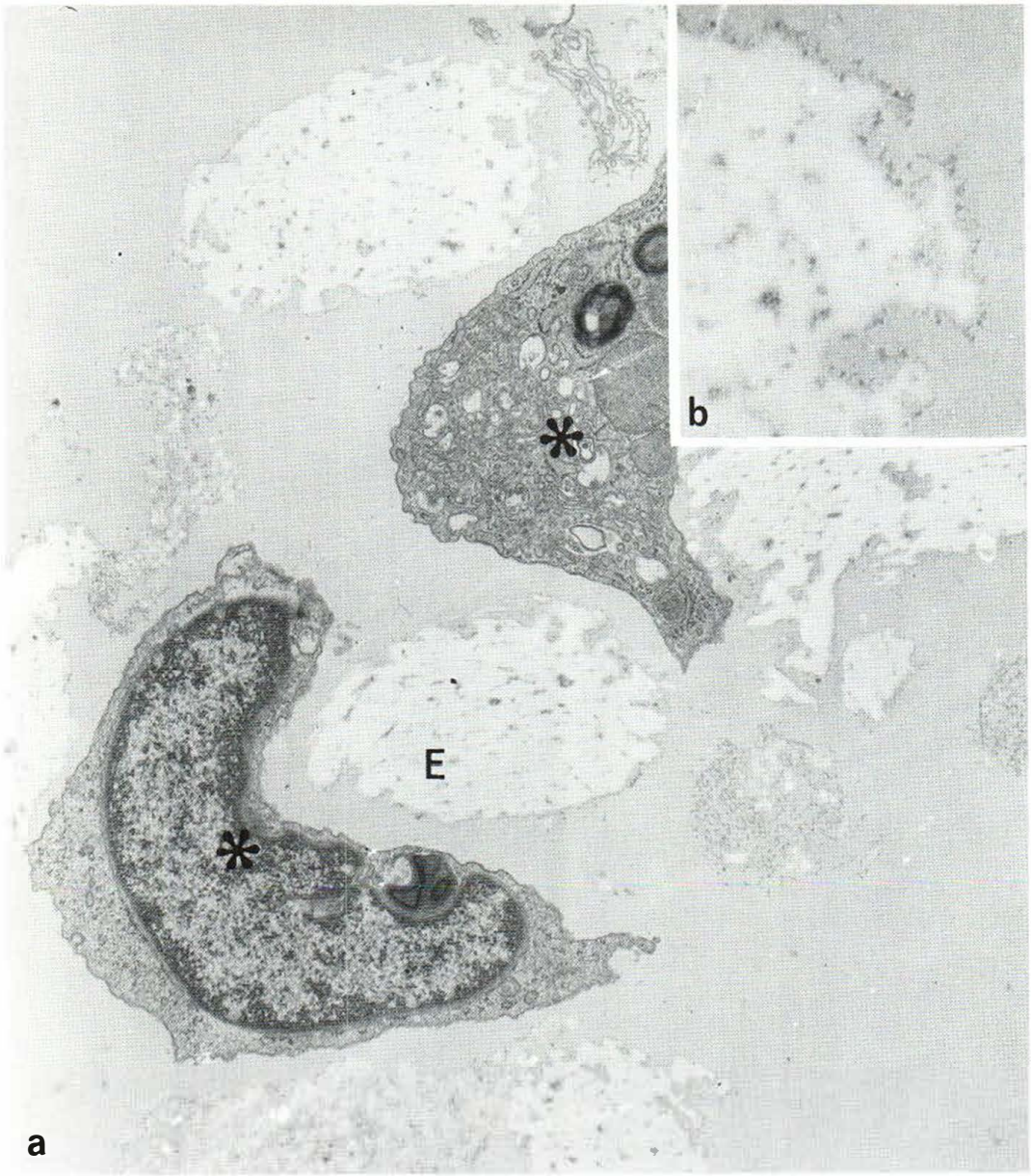

Fig. 6. (*a*) After 8 weeks of cultivation, a complete resorption of collagen fibrils is seen. Hence, the connective tissue consists of elastic fibres (*E*) and viable dermal cells (*asterisks*). ×15000. (*b*) The matrix of the elastic fibres shows no changes, while the fibrils at the periphery gradually disappear. ×60000.

found. The diameter was constant, approximately 100–120 Å across. After 3–6 weeks, few microfibrils were apparent. After 8–10 weeks, an almost complete disappearance of elastic microfibrils was noted, not only at the periphery but also within the interstices of the matrix. The predominant changes of the elastic fibres and collagen fibrils were found in the same areas of the dermis. A relative increase in the amount of elastic fibres in relation to collagen fibrils occurred during the culture period. After 6 weeks of cultivation, the subepidermal part of the dermal connective tissue consisted of elastic fibres and cells, while most collagen fibrils had disappeared (Fig. 6*a*).

III. *Proteoglycans*

The proteoglycan figures, as demonstrated by ruthenium red stained filaments with knobs, gradually decreased during the cultivation. After 2–3 weeks, a complete absence of proteoglycan figures was noticed.

CONTROLS

Two groups of controls were studied, viz. (1) explants of each individual, fixed immediately before cultivation (Fig. 3*a*); and (2) frozen and thawed explants fixed before and after maintenance in identical culture media for similar time periods (Fig. 3*b*). No connective-tissue changes like those described above were found in any of the controls.

DISCUSSION

The only previous ultrastructural study on human dermis maintained in organ culture was performed by Sakany & Gaylarde in 1970 (34). These authors maintained skin explants for 3 days and found no changes in the connective tissue. However, the present work demonstrates significant changes in dermal connective tissue after long-term cultivation. The loss of native collagen fibrils in the upper part of dermis during cultivation gives direct evidence of collagen degradation. The skin collagenase is known to be synthesized in the upper corium, while minimum amounts are produced by the epidermis and lower dermis (8). These findings tally with the location of degenerative collagen fibril changes in our explants. Most collagenases have been isolated from small tissue fragments in serum-free culture medium (15), because skin collagenase is inhibited by the α_2-macroglobulin and α_1-antitrypsin fractions of serum (9). However, in the present experiments, the resorption of collagen fibrils was a consistent feature, despite the fact that serum-enriched medium was employed. Dingle et al., 1975 (5) reported that presence or absence of the α_2-macroglobulin fraction of rabbit serum used for organ culture of cartilage tissue had no influence on the resorption of collagen. It has been suggested that the collagenolytic enzymes act only near the site of production, while the inhibitory effect of serum prevents their action at distant sites (9).

During cultivation, a collagen fibril synthesis may take place, rendering the evaluation of the morphological picture more complex. For synthesis of collagen fibrils, human fibroblast cultures require the presence of co-factors, viz. ascorbate, α-ketoglutarate, ferrous ions and molecular oxygen (11, 37). The medium used in the present study was not supplemented with ascorbic acid, hence restricting the collagen fibril synthesis. Therefore, we conclude that the morphological changes of collagen fibrils are mainly due to degradation.

Collagen fibril change A

The present study revealed two different ultrastructural aspects of collagen fibril degradation. The first event was thinning of collagen fibrils probably due to filament separation. Similar morphological observations have been reported during resorption of connective tissue in skin carrageenin granuloma (30). The collagen fibril diameters vary considerably both with individuals and with areas (44). The collagen fibril diameters of the cultivated skin explants were always compared with the control biopsies of each individual studied before cultivation. Hence, the decrease in the diameters was found to be significant. Thin collagen fibrils have been reported in fibroblast cultures (13), regenerating tendons (12), scleroderma skin (23, 33), and embryonal skin (24). In all those cases, the varying and usually thin collagen fibrils have been explained as newly formed collagen fibrils. However, similar changes have been reported in atrophic skin diseases, i.e. lichen sclerosus et atrophicus (4), necrobiosis lipoidica diabeticorum (20), and acrodermatitis chronica (38). During resorption of connective tissue in tadpole tail, a decreasing collagen fibril diameter also appeared (41). We conclude, therefore, that varying and decreasing diameters of collagen fibrils may be due to both connective tissue break-down and build-up, whereas filamentous separation has been demonstrated only in degenerating collagen fibrils.

Collagen fibril change B

Banded filamentous structures with a morphology similar to CBFA have been seen in various normal tissue, although they are more frequent in pathological tissues (6, 16, 36). In pathological skin, the CBFA have been reported in generalized scleroderma (19), scleromyxedema (3), amyloidosis and skin cancer (16, 17), lepromatous granulomas (6), leishmania granulomas (35), mycosis fungoides (21), and granuloma annulare (2).

In previous reports on nerve tissue, the CBFA-like structures were spindle-shaped and had a long-

spaced periodicity. They generally appeared in connection with the perineural collagen fibrils (6, 36). Similar structures have been observed by us in cultivated skin, but only in the vicinity of peripheral nerves, and were regarded to be distinct from the ultrastructure of the CBFA. Both structures appeared in our explants. Therefore, the morphology of perineural CBFA is not explained by any one particular method of preparation. Sun & White, 1975 (36) also emphasized the apparent difference between the CBFA-like sturcture of nerve and those found elsewhere.

It is generally suggested that the cross-banded filamentous aggregations represent fibrous long-spacing (FLS) forms of collagen. This assumption is mainly based on the work of Luse et al., 1963 (26). In an abstract form, these authors reported that FLS collagen produced *in vitro* was indistinguishable from the CBFA-like structures appearing in Schwann-cell tumours. However, the authors did not elaborate their results further. Recently, it has been shown that the periodicities of CBFA and FLS are different (16, 36).

It has been suggested that CBFA may be related to increased amounts of acid mucopolysaccharides (31). This change in the environment may produce abnormal linkage of the tropocollagen molecules. Accumulation of mucopolysaccharides at the bands of CBFA has been reported (16, 43), but this could not be reproduced in the present study.

The CBFA appeared mainly in areas where native collagen fibrils were changed. The progressive decrease in number of intact native collagen fibrils and the proportional increase in CBFA during the cultivation period, suggest that the CBFA are related to the collagen degradation. The CBFA have been demonstrated in tissues known to produce both collagenase and non-specific proteases (17). Both collagenolytic enzymes obtained from cell culture medium and bacterial collagenase applied to fresh human skin also produced CBFA (22). Collagen is degraded into small peptides by bacterial collagenase (27). Therefore, we suggest that CBFA may represent an intermediate product of enzymatically degraded collagen fibrils.

Elastic fibres and proteoglycans

In the present study, few changes were found in elastic fibres. The disappearance of elastic fibrils at the end of the cultivation period may be a degradation phenomenon. Elastase dissolves only the matrix of elastic fibres (22, 32). The elastic fibrils may be susceptible to digestion by proteolytic enzymes. Newman & Low, 1973 (28) reported that both elastic and collagen fibrils obtained from aldehyde-fixed tissue were digested by chymotrypsin at pH 7.8. However, trypsin was only slightly reactive to elastic fibrils, while the collagen fibrils were completely degraded. Enzymatic digestion of fresh unfixed connective tissue at neutral pH demonstrated that trypsin influences neither collagen nor elastic fibrils (22). The collagenolytic enzyme obtained from cell-culture medium and bacterial collagenase failed to digest both elastic matrix and fibrils of unfixed tissue (22). This is in accordance with the finding that collagenase-producing squamous cell carcinoma of skin showed resorption of collagen fibrils, while the elastic fibres remained intact (17). However, the resorption of elastic fibrils of the skin explants cannot be correlated to any specific enzyme.

Proteoglycan figures were seen less frequently during cultivation. Proteoglycan breakdown in cartilage organ culture explants, probably by lysosomal enzymes, has been reported by Dingle et al., (5). It may also take place in skin cultures. The decrease in proteoglycans may render the collagen fibril more susceptible to collagenase degradation.

Control explants

Control studies on non-viable explants cultivated in similar medium for comparable periods demonstrated that the reported findings are not a result of non-specific factors in the medium and environment (i.e. pH, temperature, ionic strength). They also demonstrated that the changes are dependent on the viability of cells, suggesting that the degrading enzymes are not stored within the cells, but are synthesized *de novo*.

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REFERENCES

1. Brandes, D. & Anton, E.: Lysosomes in uterine involution: Intracytoplasmic degradation of myofilaments and collagen. J Gerontol *24:* 55, 1969.
2. Charles, C. R., Cooper, P. H. & Helwig, E. B.: The fine structure of granuloma annulare. Lab Invest *36:* 444, 1977.
3. Danielsen, L. & Kobayasi, T.: Ultrastructural changes in scleromyxedema. Acta Dermatovener (Stockholm) *55:* 451, 1975.
4. Daróczy, J. & Török, É.: Electron microscopy of lichen sclerosus et atrophicus. Trans St Johns Hosp Dermatol Soc *60:* 183, 1974.
5. Dingle, J. T., Horsfield, P., Fell, H. B. & Barratt, M. E. J.: Breakdown of proteoglycan and collagen induced in pig articular cartilage in organ culture. Ann Rheum Dis *34:* 303, 1975.
6. Edwards, R. P.: Long-spacing collagen in skin biopsies from patients with lepromatous leprosy. Br J Dermatol *93:* 175, 1975.
7. Eisen, A. Z., Jeffrey, J. J. & Gross, J.: Human skin collagenase. Isolation and mechanism of attack on the collagen molecule. J Biochem Biophys Acta *151:* 637, 1968.
8. Eisen, A. Z.: Human skin collagenase: Localization and distribution in normal human skin. J Invest Dermatol *52:* 442, 1969.
9. Eisen, A. Z., Block, K. J. & Sakai, T.: Inhibition of human skin collagenase by human serum. J Lab Clin Med *75:* 258, 1970.
10. Eisen, A. Z., Bauer, E. A. & Jeffrey, J. J.: Human skin collagenase. The role of serum alpha-globulins in the control of activity in vivo and in vitro. Proc Natl Acad Sci USA *68:* 248, 1971.
11. Feng, J., Melcher, A. H., Brunette, D. M. & Moe, K.: Determination of α-ascorbic acid levels in culture medium: Concentrations in commercial media and maintenance of levels under conditions of organ culture. In Vitro *13:* 91, 1977.
12. Fernando, N. V. P. & Movat, H. Z.: Fibrillogenesis in regenerating tendon. Lab Invest *12:* 214, 1963.
13. Goldberg, B. & Green, H.: An analysis of collagen secretion by established mouse fibroblast lines. J Cell Biol *22:* 227, 1964.
14. Gross, J. & Lapiere, C. M.: Collagenolytic activity in amphibian tissues: A tissue culture assay. Proc Natl Acad Sci USA *48:* 1014, 1962.
15. Harris, E. D. & Krane, S. M.: Collagenases. N Engl J Med *295:* 557, 1974.
16. Hashimoto, K. & Okyama, H.: Cross-banded filamentous aggregation in the human dermis. J Invest Dermatol *62:* 106, 1974.
17. Hashimoto, K., Yamanishi, Y., Maeyens, E., Dabbous, M. K. & Kanzaki, T.: Collagenolytic activities of squamous cell carcinoma of the skin. Cancer Res *33:* 2790, 1973.
18. Hentzer, B. & Kobayasi, T.: Intracellular collagen fibrils in cultured human skin. Acta Dermatovener (Stockholm) (in press).
19. Kobayasi, T. & Asboe-Hansen, G.: Ultrastructure of generalized scleroderma. Acta Dermatovener (Stockholm) *52:* 81, 1972.
20. Kobayasi, T., Danielsen, L. & Asboe-Hansen, G.: Ultrastructure of necrobiosis lipoidica diabeticorum. Acta Dermatovener (Stockholm) *54:* 427, 1974.
21. Kobayasi, T., Hentzer, B. & Asboe-Hansen, G.: Ultrastructural histogram of cells of mycosis fungoides infiltrate. J Ultrastruct Res *57:* 234, 1976.
22. Kobayasi, T., Hentzer, B. & Asboe-Hansen, G.: Degradation of dermal fibrillar structures: Effects of collagenase, elastase, dithioerythritol and citrate. Acta Dermatovener (Stockholm) *57:* 379, 1977.
23. Korting, G. W., Holzmann, H. & Forssmann, W. G.: Zur Frage der Häufigkeitsverteilung der Kollagenfibrillendecken bei einigen Bindegewebskrankheiten. Arch Klin Exp Dermatol *223:* 105, 1965.
24. Lenke, K. W.: Elektronenmikroskopische Untersuchung über die Differenzierung der Interzellarsubstanz der menschlichen Lederhaut. Z Zellforsch *42:* 331, 1955.
25. Luft, J. H.: Improvements in epoxy resin embedding methods. J Biophys Biochem Cytol *9:* 409, 1961.
26. Luse, S. A., Zoph, D. & Cox, J. W.: An electron microscopic study of in vitro and in vivo long-spacing collagen. Anat Rec *145:* 254, 1963.
27. Mandl, J.: Collagenases and elastases. Adv Enzymol (Interscience Publication, New York) *23:* 163, 1961.
28. Newman, T. L. & Low, F. N.: The effect of enzymes on extracellular connective tissue components in the developing chick aorta. Am J Anat *136:* 407, 1973.
29. Parakkal, P. F.: Involvement of macrophages in collagen resorption. J Cell Biol *41:* 345, 1969.
30. Pérez-Tanayo, R.: Collagen resorption in carrageenin granulomas. II. Ultrastructure of collagen resorption. Lab Invest *22:* 142, 1970.
31. Ramsey, H. J.: Fibrous long-spacing collagen in tumors of the nervous system. J Neuropathol Exp Neurol *24:* 40, 1965.
32. Ross, R. & Bornstein, P.: The elastic fiber. I. The separation and partial characterization of its macromolecular components. J Cell Biol *40:* 366, 1969.
33. Rupec, M. & Braun-Falco, O.: Elektronenmikroskopische Untersuchungen über das Verhalten der Kollagenfibrillen der Haut bei Sklerodermie. Arch Klin Exp Dermatol *218:* 543, 1964.
34. Sarkany, I. & Gaylarde, P. M.: Ultrastructural changes in human skin maintained in organ culture. Br J Dermatol *83:* 572, 1970.
35. Sandbank, M.: Cross-striated banded structures in cutaneous leishmaniasis. Br J Dermatol *96:* 653, 1977.
36. Sun, C. N. & White, H. J.: Extracellular cross-striated banded structures in human connective tissue. Tissue Cell *7:* 419, 1975.
37. Switzer, B. R. & Summer, G. K.: Collagen synthesis in human skin fibroblasts: Effects of ascorbate, α-ketoglutarate and ferrous ion on proline hydroxylation. J Nutr *102:* 721, 1972.
38. Teller, H.: Elektronenmikroskopische Untersuchungen des Bindegewebes bei Hautatrophie. Arch Klin Exp Dermatol *206:* 730, 1957.
39. Ten Cate, A. R.: Morphological studies of fibrocytes

in connective tissue undergoing rapid remodelling. J Anat *112:* 401, 1972.
40. Thiéry, J.-P.: Mise en evidence des polysaccharides sur coupes fines en microscopie electronique. J Microsc *6:* 987, 1967.
41. Usuku, G. & Gross, J.: Morphologic studies of connective tissue resorption in the tail fin of metamorphosing bullfrog tadpole. J Dev Biol *11:* 352, 1965.
42. Weissmann, G. & Fell, H. B.: The effect of hydrocortisone on the response of fetal rat skin in culture to ultraviolet irradiation. J Exp Med *116:* 365, 1962.
43. Yardley, J. H. & Brown, G. D.: Fibroblasts in tissue culture. Use of colloidal iron for ultrastructural localization of acid mucopolysaccharides. Lab Invest *14:* 501, 1965.
44. Zelickson, A. S.: Ultrastructure of Normal and Abnormal Skin. Lea & Febiger, Philadelphia, p. 242, 1967.

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B. Hentzer, M.D.
Department of Dermatology, Rigshospital, Blegdamsvej 9
DK-2100 Copenhagen
Denmark