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Focal Dermal Hypoplasia: Abnormal Growth Characteristics of Skin Fibroblasts in Culture

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Focal dermal hypoplasia is an inherited disease affecting the skin, bones, ocular and dental structures. Histologically, the skin lesions are characterized by a marked decrease in dermal connective tissue. Fibroblast cultures initiated from both the skin lesions and the unaffected skin from a patient with focal dermal hypoplasia were used to assess the growth characteristics of these cells as well as potential abnormalities in the metabolism of collagen, the major extracellular gene product of skin fibroblasts. Cells from affected skin were characterized by a markedly compromised growth potential with a mean population doubling time twice that of the controls and a final saturation density of one-fifth that of control cultures. Phase contrast microscopy revealed that fibroblasts derived from the skin lesions were strikingly abnormal and characterized by a large granular cytoplasm with cytoplasmic vacuoles. Despite these findings, the rate of collagen synthesis, measured as the formation of [^{3}H]hydroxyproline in relation to DNA and cell protein was undisturbed in focal dermal hypoplasia. Furthermore, the relative synthesis of genetically distinct procollagens of type I and III, isolated by DEAE-cellulose chromatography, was unaltered in the affected cell strains. These findings indicate that, although the synthesis of collagen by individual fibroblasts is normal, an abnormality in cell kinetics may have relevance to the absence of collagen and other connective tissue components of the dermis in focal dermal hypoplasia.

Focal dermal hypoplasia is a syndrome characterized by widespread anomalies of cutaneous, osseous, ocular and dental structures. The syndrome, as defined by Goltz et al [2,3], consists of a congenital decrease in the dermal connective tissue in association with other defects involving tissues of ectodermal and mesodermal origin. This syndrome is predominantly seen in females, a fact probably explained by an X-linked dominant inheritance, so that the heterozygous females live to demonstrate the congenital abnormalities. In the hemizygous males, however, it has been postulated that the disease is so severe that intrauterine death occurs.

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Abbreviations:
DEAE: diethylaminoethyl
DMEM: Dulbecco's Modified Eagle's Medium
HEPES: N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid
Na_2EDTA: disodium ethylenediaminetetraacetate

Histopathologically the lesions are characterized by the absence of dermal connective tissue, so that mature fat cells impinge directly upon the epidermis, separated only by a thin layer of reticular fibers [4,5]. Although focal dermal hypoplasia is usually associated with multiple defects in the affected individual, several cases have been reported with clinical findings limited to the skin [2,6–8]. The molecular basis for the absence of connective tissue in focal dermal hypoplasia is unknown; however, we postulated that the cutaneous abnormalities in this disease might result either from an aberration in the capacity of the cellular elements of the connective tissue to synthesize the major extracellular matrix proteins or because these cells might display altered growth properties. In the present study cultured skin fibroblasts from a patient with focal dermal hypoplasia, who demonstrated typical skin lesions associated with minimal dental anomalies, have been employed to examine the growth characteristics both in the affected and normal skin. In addition, these same cells have been used to characterize the synthesis of the major extracellular gene product, collagen.

MATERIALS AND METHODS

Clinical

The patient is a 23-yr-old female with normal intelligence, the product of a full-term pregnancy and uncomplicated delivery. There was no familial history of consanguinity, miscarriage, or of skin, skeletal, ocular or dental anomalies. The patient has 2 normal male siblings. At approximately 6 weeks of age, scaly, erythematous skin changes were noted on the patient's chin and knees. No blisters, papules or erosions were present. The erythema on the chin faded over the next several months, but the erythema on the knees spread both proximally and distally over the legs. During the next 3–4 mo, the lesions extended to involve the anterior axillary folds. The skin changes have remained constant since age 4. The patient's general growth and development have been normal.

On physical examination the abnormalities were found to be limited to the cutaneous and dental findings. The skin lesions were erythematous with telangiectasia, hyperpigmentation and atrophy (Fig 1). The lesions had a reticulated, cribriform pattern and followed a linear zosteriform distribution on both buttocks. One area of involvement was located on the left posterior thigh which extended to the posterior lower leg while the other lesion originated on the buttocks and was distributed around the anterior thigh and the anterior aspect of the lower leg. The patient had similar hyperpigmented, telangiectatic, and atrophic lesions over both anterior axillary lines extending down both arms. The histology of these lesions was consistent with focal dermal hypoplasia (see below). The patient had several perianal papillomas. There were no keratotic papules or areas of fat herniation. The patient's nails, scalp and hair were normal.

Dental examination showed a torus palatinus and a small area (1 mm × 1 mm) of enamel hypoplasia on the labial surface of the mandibular left central incisor. The mandibular dental midline was shifted to the patient's left approximately 3 mm. Ophthalmologic examination was normal.

Laboratory studies including a complete blood count, urinalysis, SMA-6, SMA-12, serum protein and immunoelectrophoresis were all within normal limits. An audiogram, EEG, and EKG were normal. An intravenous pyelogram and dental panorex examination were normal. A complete skeletal survey was normal, and specifically, there was no osteopathia striata. Chromosome studies by the fluorescein labeling technique of cultured skin fibroblasts and peripheral leukocytes showed a normal female karyotype.

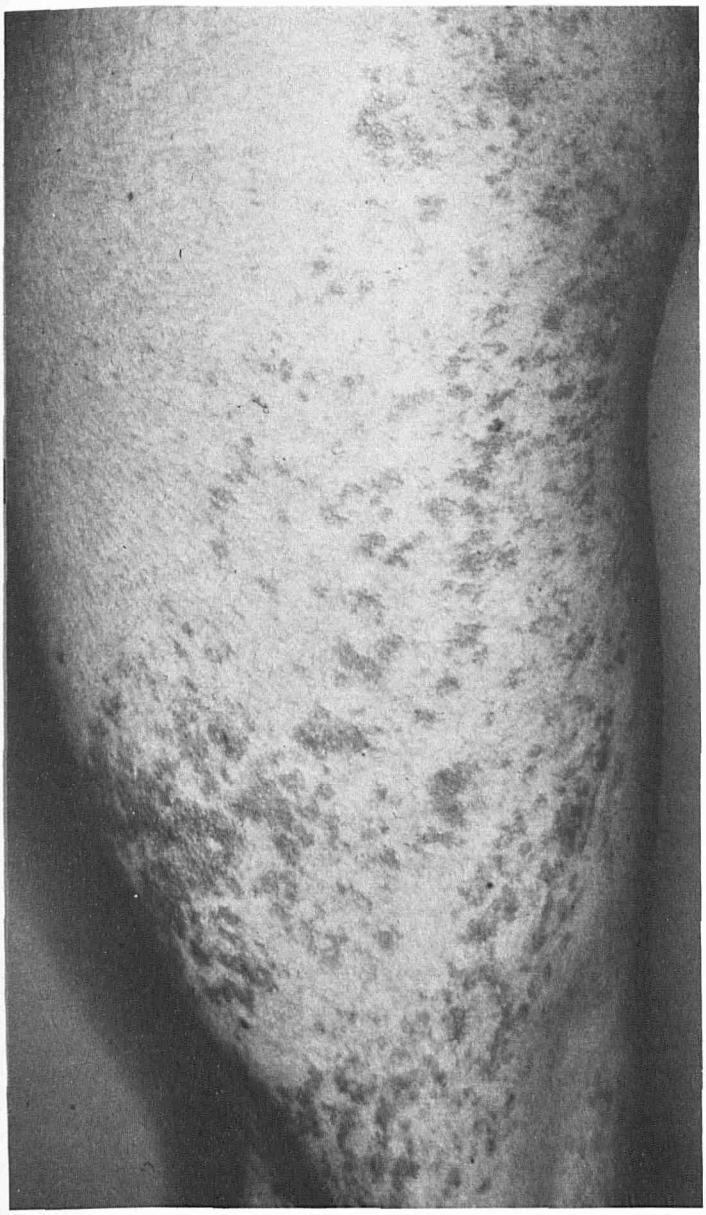

FIG 1. Clinical presentation of the cutaneous abnormalities in the patient with focal dermal hypoplasia. The skin lesions are characterized by atrophy, erythema, telangiectasia, and hypo- and hyperpigmentation.

Histology

Multiple skin biopsies for light microscopy were taken under local anesthesia with 1% lidocaine, after obtaining informed consent. The skin specimens were fixed in 10% neutral formalin, and processed routinely. The sections were stained with hematoxylin-eosin, Trichrome-Masson, orcein-Giemsa, iron-gallein, and PAS (with and without digestion with diastase for 30 min at 37°C). Histologic examination of the skin revealed a normal epidermis (Fig 2). The dermis was markedly decreased in thickness, but the collagen in the deep dermis was normal appearing (Fig 2 and 3). The blood vessels in the upper and mid-dermis were accentuated by a dense cuffing of mature adipose cells. An abnormally thick cushion of adipocytes was also seen around the sweat glands. Small capillaries located in the superficial dermis and especially in the dermal papillae, exhibited the same phenomenon. Approximately two-thirds of the collagenous dermis was replaced by adipose tissue that did not appear to herniate.

For histologic staining of cultured skin-fibroblasts, sterilized microscope glass slides were placed in the cell culture flasks, and the fibroblasts were allowed to grow in a monolayer culture on the slides. The slides were fixed in neutral formalin and stained with hematoxylin-eosin, phosphotungstic acid-hematoxylin, and PAS, as described above. Some slides were examined for lipids without prior fixation by staining with oil red 0 (see Results).

Fibroblast cultures

Primary fibroblast cultures were initiated from 3-mm skin punch biopsies of the lesion and adjacent normal-appearing skin from the patient as well as from age-matched control subjects. Cells were subcultivated in Dulbecco's Modified Eagle's Medium + glutamine (DMEM) with 30 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer, pH 7.6, and containing 20% fetal calf serum, 200 units per ml of penicillin and 200 μg per ml of streptomycin. The cells were passed serially by trypsinization and they were used in the second to fifth passage. The efficiency of cellular attachment was assessed 24 hr after the cultures were seeded and was 87.7 ± 24.5% (mean ± SE) in the control cell strains compared to 93.9 ± 0.6% in the cells from the affected skin of the focal dermal hypoplasia patient (p = NS).

The growth kinetics of the fibroblasts were determined by seeding parallel cultures from a single pool at low density. Replicate cultures were fed serum-containing medium every 2 days until they reached the saturation density. Duplicate cultures were harvested daily for cell counts.

Collagen Biosynthesis

To measure the rate of procollagen synthesis, the fibroblasts grown on a 75 cm^2 flask (Falcon Plastics, Oxnard, California) into early confluency were incubated with 30 μCi [^{3}H]proline in 5.0 ml of DMEM containing 20% dialyzed fetal calf serum in HEPES buffer, as described above. Ascorbic acid, 50 μg/ml, and β-aminopropionitrile, 20 μg/ml, were added to the medium 4 hr prior to the labeling [9]. At the end of a 20 hr labeling period, the incubation medium was removed and a mixture of protease inhibitors was added to give the final concentrations of 10 mM N-ethylmaleimide, 20 mM Na_2EDTA, and 1 mM α-toluenesulfonyl fluoride. The cells were scraped with a rubber policeman into 5 ml of 0.4 M NaCl, 0.1 M Tris-HCl, pH 7.5, containing the same protease inhibitors as above and then sonicated at 60 Hz for 30 sec. Aliquots of

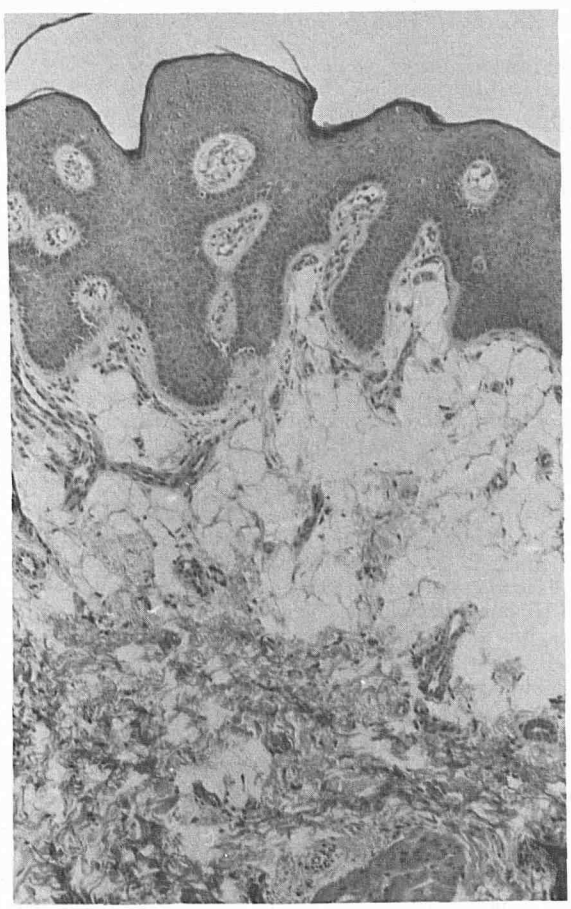

FIG 2. Histology of the lesions in focal dermal hypoplasia. Note the absence of connective tissue in the upper dermis, the collagen fibers being replaced by adipose tissue (H & E, × 150).

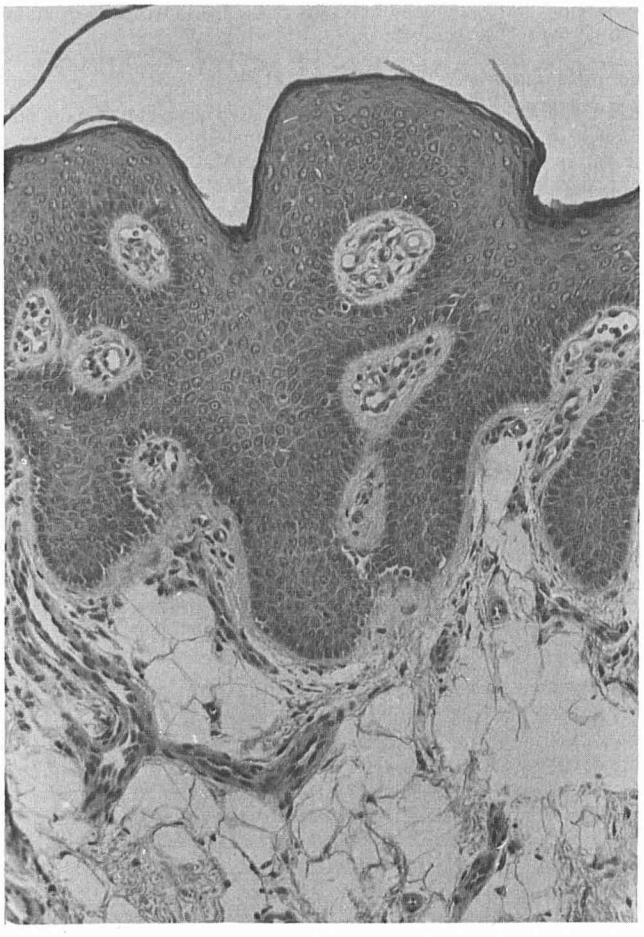

FIG 3. A detail of Fig 2, demonstrating adipose tissue in the papillary dermis separated from the normal-appearing epidermis by a narrow band of connective tissue (H & E, × 250).

the medium and cell fractions were dialyzed against running tap water, hydrolyzed in 6 M HCl, and the [^{3}H]hydroxyproline was assayed using a specific radiochemical method (10). Aliquots of the cell fraction were also dialyzed against 0.15 M NaCl, 0.001 M Tris-HCl, pH 7.4, and taken for assay of total cell protein [11] and DNA [12]. The amount of radioactive hydroxyproline synthesized by the cultures, in relation to DNA and cellular protein, was taken as an index of procollagen synthesis.

The synthesis of genetically distinct procollagens was also studied by incubating the cells with radioactive proline, as indicated above. At the end of the incubation period, protease inhibitors (see above) were added to the medium, and [^{3}H]procollagen was partially purified by the precipitation with 114 mg ammonium sulfate per ml (20% saturation). The precipitate was dissolved in 5 ml of 0.025 M Tris-HCl, pH 7.5, containing 2 M urea and 1 mM Na_2EDTA, and type I and type III procollagens were separated by DEAE-cellulose chromatography using a linear gradient from 0 to 0.22 M NaCl, as described elsewhere [13,14]. Fractions eluted from the column were counted using a Beckman LS 119 liquid scintillation counter.

RESULTS

Fibroblast cultures were initiated from the affected and unaffected areas of skin to examine the kinetics of cell growth under tissue culture conditions. Fibroblasts derived from the lesions were strikingly abnormal by phase contrast microscopy at early passage and were characterized by a large granular cytoplasm and prominent cytoplasmic vacuoles (Fig 4). These changes were less frequently observed with each cell culture passage. The vacuoles were seen only in fibroblast cultures from the affected skin but not in those from the unaffected skin or in control skin fibroblast cultures. Attempts to identify the contents of the cytoplasmic granules by histologic staining methods yielded inconclusive results. Specifically, the material in the granules appeared to be PAS-negative and it did not stain for lipid with oil red 0.

Detailed measurements of the growth kinetics of these cells demonstrated that the mean population doubling time of cells in cultures initiated from the lesions was about twice as long as observed in cultures derived from unaffected skin of the same patient, or from healthy controls (Table I). More strikingly, despite maintenance of the cultures for several weeks with feeding every other day, the final saturation density of the cell cultures (i.e., at confluence) from the affected skin was one-fifth that of the controls (Table II). These findings indicate that the cells in the affected skin are characterized by a significantly compromised growth potential. Neither the growth kinetics nor the saturation density changed with passage of the focal dermal hypoplasia cells.

In further studies, the rate of procollagen synthesis, measured as the formation of radioactive hydroxyproline when the fibroblasts were incubated with [^{3}H]proline, was also determined. The results demonstrated that the rate of hydroxyproline formation, when expressed in relation to DNA, was not different in cell strains derived from affected and unaffected skin. In addition, comparison of the rate of [^{3}H]hydroxyproline synthesis with control cell strains revealed no differences (Table III). Similar results were obtained when hydroxyproline synthesis was expressed in relation to cell protein. Thus, the synthesis of procollagen, the major extracellular connective tissue component of the skin, was not changed in the cultured fibroblasts from the patient studied here.

The synthesis of genetically distinct types of procollagen was also studied by incubating the cells with radioactive proline; type I and type III [^{3}H]procollagens were then separated by DEAE-cellulose chromatography. The results demonstrated that these 2 types of procollagens were synthesized in a normal ratio (Fig 5). More specifically, both precursor forms of collagen were synthesized in an approximate 5:1 ratio, a relationship which was also observed in the control fibroblasts here (Fig 5*A*) as well as in our previous studies (see reference 15).

DISCUSSION

In the present study we have demonstrated that skin fibroblasts from a patient with focal dermal hypoplasia (the Goltz syndrome) display markedly abnormal growth characteristics under tissue culture conditions. The affected cells were noted to have abnormally long mean population doubling times, and the final saturation density of the affected cultures was only one-fifth that of the controls. These abnormal growth characteristics were observed only in the fibroblasts obtained from the skin lesions, while the cells from the normal-appearing skin of the same patient behaved in a similar fashion as cells obtained from normal controls. In addition to the abnormal growth kinetics, the affected cells demonstrated peculiar cytoplasmic vacuoles which were not seen in the unaffected skin or in the control fibroblasts. Thus, it appears that fibroblasts in the lesions of focal dermal hypoplasia represent a distinct population, and the patchy distribution of these cells in the skin of affected females may reflect mosaicism as a consequence of the lyonization process [16].

Of particular interest is a recent report [17] suggesting that human skin fibroblasts cultured from the papillary dermis exhibit greater proliferative capacities *in vitro* than fibroblasts from the reticular dermis. Based on this observation the decreased proliferative capacity of the fibroblasts in the lesion of focal dermal hypoplasia might be reflective of a source of the cells in the reticular dermis. Such a possibility appears very unlikely for at least 2 reasons. First, the observed differences in the growth characteristics between reticular and papillary fibroblasts reported previously [17] are significantly smaller than the differences observed in the present case. Secondly, the

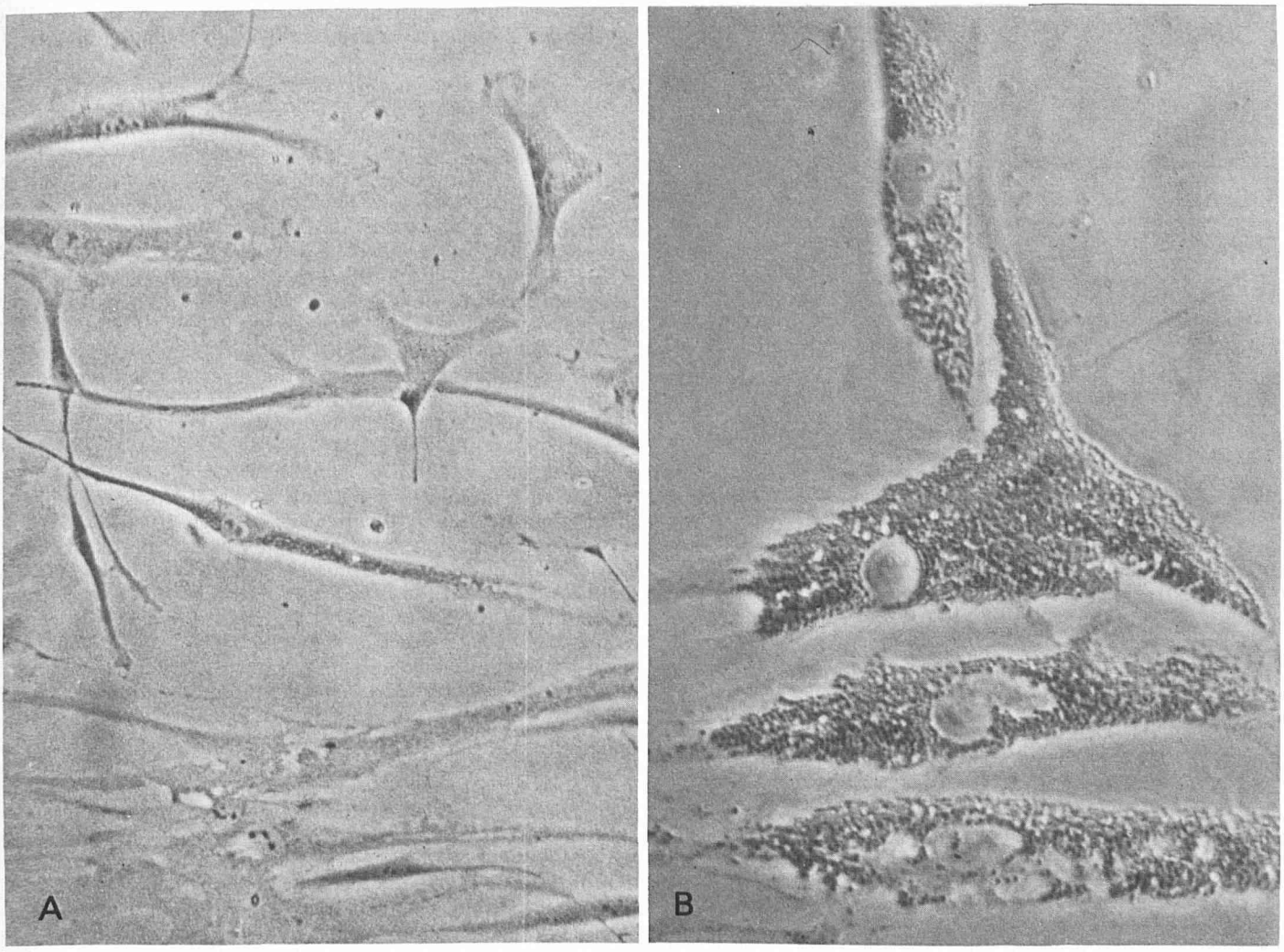

FIG 4. Morphology of the fibroblasts cultured from skin in focal dermal hypoplasia. The cells from the skin lesions in the second passage (*B*) are characterized by a granular cytoplasm and large cytoplasmic vacuoles which are not seen in the cells from normal appearing skin or in the controls (*A*). (Phase contrast, × 600).

TABLE 1. *Growth kinetics of normal and focal dermal hypoplasia fibroblasts*

Culture	Population doubling time
	Days[a]
Controls[b]	1.23 ± 0.16
FDH, unaffected skin	1.43 ± 0.21[c]
FDH, affected skin	2.88 ± 0.66[d]

[a] Expressed as mean ± SE.
[b] Controls represent values from 3 different normal human skin fibroblast lines.
[c] p = NS compared to the control cultures.
[d] $p < 0.01$ compared to the control cultures.

TABLE II. *Saturation density of normal and focal dermal hypoplasia fibroblasts in culture*

Culture	Saturation density
	Cells × 10^3/*25cm*^{2} *flask*[a]
Controls[b]	2,330 ± 646
FDH, unaffected skin	2,335 ± 15[c]
FDH, affected skin	440 ± 66[d]

[a] Expressed as mean ± SE.
[b] Controls represent values from 2 different normal human skin fibroblast lines.
[c] p = NS compared to the control cultures.
[d] $p < 0.025$ compared to the control cultures.

TABLE III. *The rate of procollagen synthesis by cultured skin fibroblasts*

Culture	[^{3}H]Hydroxyproline[a] (dpm × 10^{-2}/μg DNA/hr)
Controls[b]	14.2 ± 3.5
FDH, unaffected skin	11.3 ± 1.5[c]
FDH, affected skin	12.1 ± 2.5[c]

[a] Expressed as mean ± SE of 4 parallel determinations.
[b] Controls represent values from 3 different normal human skin fibroblast lines.
[c] p = NS compared to control cultures.

morphology of the cells derived from the reticular and papillary dermis is not strikingly different, and cells with abnormal vacuoles, such as reported here, are not seen in control cultures initiated either from full-thickness or split-thickness skin biopsies. It appears, therefore, that the abnormalities observed in the growth kinetics and morphology of the fibroblasts in the lesions of focal dermal hypoplasia reflect an inherent cellular defect in a structure essential for normal cell proliferation; such a defect might, for example, reside in the membrane structures of the cells.

Since collagen is the major extracellular component of the dermis [18], it was initially postulated that abnormal synthesis of procollagen by the affected cells could explain the absence of

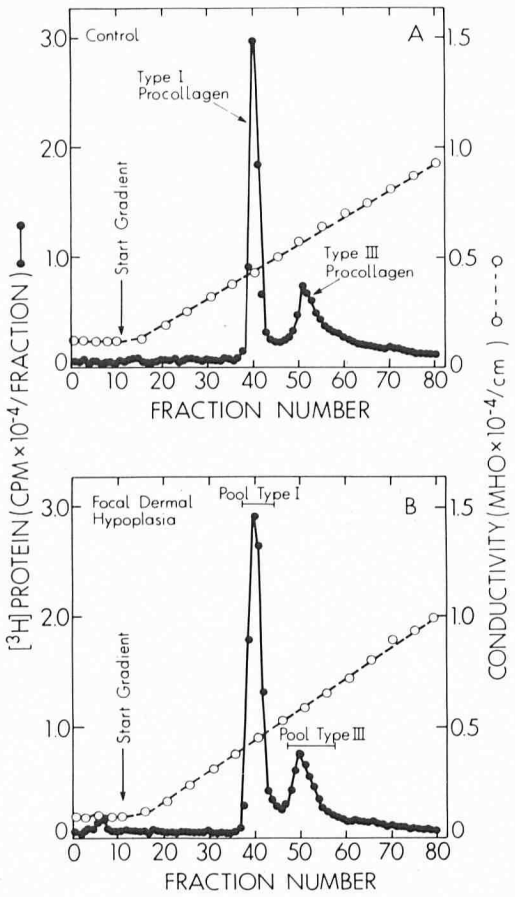

FIG 5. Isolation of type I and type III procollagens synthesized and secreted by cultured skin fibroblast. The cell cultures were incubated in the presence of [^{3}H]proline for 20 hr, and the medium ^{3}H-protein was chromatographed on DEAE-cellulose, as described in Materials and Methods. The peaks of radioactivity containing type I and type III [^{3}H]procollagens were pooled as indicated by the horizontal bars, and the respective procollagens were quantitated by assaying the [^{3}H] hydroxyproline content of the peaks. *A*: ^{3}H-labeled protein in the medium of control cell cultures; *B*: ^{3}H-labeled protein in the medium of fibroblast cultures from the affected skin in focal dermal hypoplasia.

connective tissue. Such a hypothesis was reasonable, since several clinical conditions affecting the skin and other connective tissues have been reported to involve defects in the biochemistry of collagen [18,19]. Contrary to our expectations, the rate of collagen synthesis by the affected fibroblasts was unaltered when the values were related to the DNA or cell protein content of the cultures. In addition, the relative synthesis of genetically distinct procollagens of type I and type III was not changed in the affected cells. These observations further strengthen the suggestion that an abnormality in the growth kinetics, rather than in the synthetic capacity of the fibroblasts might be responsible for the absence of collagen and other connective tissue components in the dermis in focal dermal hypoplasia.

Although the patient studied by us demonstrated typical cutaneous manifestations and the diagnosis was confirmed by microscopic examination of the skin, this case is somewhat unusual in that the multiplicity of stigmata, such as mental retardation, as well as gross ocular and osseous anomalies frequently associated with this syndrome were lacking. In fact, this patient's findings were limited to minor dental anomalies in addition to the typical cutaneous findings. Upon reviewing the current world literature we found 4 cases reported with a limited form of the focal dermal hypoplasia syndrome [2,6–8]. Features of these cases as well as the case presented here are shown in Table IV. It can be noted that all these 5 cases have similar cutaneous findings of telangiectasia, atrophic indentations of the skin, and hyper- and hypopigmentation. The lesions occur in a linear, reticular, and zosteriform pattern on the extremities and the trunk. Histologically, they all show fat cells lying near the epidermis characteristic of focal dermal hypoplasia. Finally, in all these cases there is an absence of other major anomalies, with the manifestations of the focal dermal hypoplasia syndrome being largely limited to the cutaneous findings. On the basis of these considerations we conclude that the patient presented in our study had a limited form of focal dermal hypoplasia. It may well be that such a limited form represents one end of the spectrum of severity of the disease, or alternatively, patients with the limited form may represent a distinct subgroup or subset of the focal dermal hypoplasia syndrome.

It should be emphasized that there are 2 diseases that should be considered in the differential diagnosis of focal dermal hy-

TABLE IV. *Features of patients with limited form of focal dermal hypoplasia*

Reference	Cutaneous Manifestations	Histology	Other Features
Jessner, 1921 [6]	Atrophy, telangiectasia, hypopigmentation, hyperpigmentation	Atrophy of epidermis, plasma cell infiltrates around enlarged papillary vessels, decreased elastic fibers of papillary net	—
Gertler, 1954 [7]	Atrophy, telangiectasia, hyperpigmentation, atrophic indentations of the skin, lichenoid papules on the thigh	Thinned dermis with fat cells, lying near the epidermis, variable width of collagen fibers which stain irregularly	—
Pincelli, 1957 [8]	Atrophy, telangiectasia, reticular hyperpigmentation, ulcer on the foot, linear, zosteriform pattern	Thinned dermis with fat cells lying near the epidermis	EKG showed a functional alteration of conduction; EEG showed a light diffuse arrhythmia without localization and thought to be of no pathologic significance
Goltz et al, 1962 [2, case 1]	Atrophic indentations of the skin, hypo- and hyperpigmentation, scalp hair growth decreased, linear pattern on the extremities, subcutaneous fat herniations	Adipose tissue in mid-corium and in some places lying almost directly below the epidermis	Born with a generalized, scaling, erosive dermatosis; dystrophic fingernails and toenails
The present case	Atrophic indentations of the skin, hyper- and hypopigmentation, telangiectasia, reticular, linear and zosteriform pattern, perianal papillomas	Thinned dermis with fat cells lying near the epidermis and surrounding the adnexal structures	Enamel hypoplasia; mandibular asymmetry

poplasia, namely nevus lipomatosus cutaneous superficialis of Hoffmann-Zurhelle and congenital poikiloderma of Rothmund-Thomson. These 2 conditions are, however, clearly distinct entities and can readily be separated from focal dermis hypoplasia. First, nevus lipomatosus is usually manifested in early adult life, is not generalized, is characterized by fat herniations, and is not associated with any major anomalies. Secondly, poikiloderma of Rothmund-Thomson, although characterized by atrophy, telangiectasia, and hyper- and hypopigmentation of the skin, can be distinguished clinically from focal dermal hypoplasia by the presence of light sensitivity, premature development of solar keratoses, and cutaneous malignancies. Also, histologically the epidermal changes, such as hyperkeratosis, parakeratosis and hydropic degeneration of the basal layer, and dermal changes, such as vascular proliferation, are not seen in focal dermal hypoplasia.

We conclude that the patient studied here had a limited form of focal dermal hypoplasia and that the abnormal growth kinetics of the fibroblasts could explain the absence of connective tissues in the skin of this patient. Efforts are currently underway to test this hypothesis in other patients with focal dermal hypoplasia.

REFERENCES

1. Uitto J, Bauer EA, Santa-Cruz DJ, Loewinger RJ, Eisen AZ: Skin fibroblasts in focal dermal hypoplasia: Growth characteristics and collagen metabolism in culture. J Invest Dermatol 72:199, 1979
2. Goltz RW, Peterson WC, Gorlin RJ, Ravits HG: Focal dermal hypoplasia. Arch Dermatol 86:708–717, 1962
3. Goltz RW, Henderson RR, Hitch JM, Ott JE: Focal dermal hypoplasia syndrome. A review of the literature and report of two cases. Arch Dermatol 101:1–11, 1970
4. Howell JB: Nevus angiolipomatosus versus focal dermal hypoplasia. Arch Dermatol 92:238–248, 1965
5. Lever WF, Schaumburg-Lever G: Histopathology of the skin, 5th edition. JB Lippincott Company, Philadelphia, 1975, pp 67 and 68
6. Jessner M: Case Report. Verhandlungen der Breslauer dermatologischen Vereinigung. Arch Derm Syph 133:48, 1921
7. Gertler W: Poikiloderma congenitale (Thomson). Derm Wochenschrift 130:1013–1015, 1954
8. Pincelli L: Contributo allo studio delle poichilodermie (a proposito di un caso di poichilodermia congenita a carattere nevico). Arch Ital Derm 29:33–53, 1957
9. Booth BA, Polak KL, Uitto J: Collagen biosynthesis by human skin fibroblasts. I. Optimization of the culture conditions for synthesis of type I and type III procollagens. Biochim Biophys Acta, 607: 145–160, 1980.
10. Juva K, Prockop DJ: Modified procedure for the assay of H^3- or C^{14}-labeled hydroxyproline. Analyt Biochem 15:77–83, 1966
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951
12. Burton K: A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 62:315–323, 1956
13. Lichtenstein JR, Byers PH, Smith BD, Martin GR: Identification of the collagenous proteins synthesized by cultured cells from human skin. Biochemistry 14:1589–1594, 1975
14. Uitto J, Lichtenstein JR, Bauer EA: Characterization of procollagen synthesized by matrix-free cells isolated from chick embryo tendons. Biochemistry 15:4935–4939, 1976
15. Uitto J, Bauer EA, Eisen AZ: Scleroderma: Increased biosynthesis of triple-helical type I and type III procollagens associated with unaltered expression of collagenase by skin fibroblasts in culture. J Clin Invest 64:921–930, 1979
16. McKusick VA: Heritable Disorders of Connective Tissue, 4th edition. The CV Mosby Company, Saint Louis, 1972, pp 10 and 11
17. Harper RA, Grove G: Human skin fibroblasts derived from papillary and reticular dermis: Differences in growth potential in vitro. Nature 204:526–527, 1979
18. Bauer EA, Uitto J: Collagen in cutaneous diseases. Int J Dermatol 18:251–270, 1979
19. Uitto J, Lichtenstein JR: Defects in the biochemistry of collagen in diseases of connective tissue. J Invest Dermatol 66:59–79, 1976