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Dermatosparaxis in a Himalayan Cat: I. Biochemical Studies of Dermal Collagen

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Dermatosparaxis, a genetic disease, results from the deficiency of the NH_2 procollagen peptidase, an enzyme which removes the NH_2-terminal nontriple-helical extensions from procollagen. We have identified a Himalayan cat which has deficient amino terminal procollagen peptidase activity. The partially processed precursor chains pNα1 (110,000 daltons) and pNα2 (99,000 daltons) were identified by sodium dodecyl sulfate electrophoresis. In contrast to that from a normal animal, the 20,000 *xg* supernatant of a skin homogenate failed to convert pNcollagen to collagen. Amino acid analysis of pNα1 and pNα2 chains demonstrated the presence of cysteine and a lower percentage of hydroxyprolyl and glycyl residues due to the presence of the amino terminal extensions. The disorder in this animal is milder than that in sheep and cattle which is reflected in the longer survival and relatively smaller proportion of pNα chains in skin. The defect was also demonstrated by skin fibroblasts in culture.

Dermatosparaxis is an inherited disease of cattle [1] and sheep [2,3] which is the result of a deficiency of the NH_2-terminal procollagen peptidase, the enzyme which cleaves the amino-terminal nonhelical extension from the precursor procollagen molecule [4–6]. Although procollagen has both carboxyl and amino-terminal extensions [7-9] which are cleaved to yield collagen, procollagen with the amino terminus (or pN-collagen) is the only precursor that accumulates in dermatosparaxic animals. These findings initially suggested existence of at least 2 procollagen peptidases, the carboxyl and amino terminal enzymes; this hypothesis has been confirmed by the recent separation of these enzymes [10].

A major difference in the reported dermatosparaxic diseases is the severity of the enzymatic deficiency. The sheep have almost no collagen α chains present in skin [6] whereas cattle [4] have significant amounts of normal chains present. Here we report the biochemical findings in a cat which has a relatively mild disorder judging from the amounts of precursor chains present and from the clinical picture.

MATERIALS AND METHODS

Animal Biopsy

A local veterinarian referred to us a 6-mo-old female Himalayan cat which he believed had abnormally fragile skin. Upon examination, the skin of the cat was noted to be extremely fragile, hyperextensible, and was velvety and thin.

At 18 mo of age skin biopsies were obtained from the affected cat and an age and sex-matched control. The cats were anesthetized with Thiamylol and the hair over the left shoulder was clipped. A 4-cm square piece of skin was removed from an area which had no noticeable scars, and the subcutaneous fat was dissected away. Most of the biopsy was frozen and stored in liquid nitrogen; one portion was explanted and fibroblasts were grown.

Sample Preparation for Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

The frozen skin samples were pulverized with a steel mortar and pestle at liquid nitrogen temperatures and homogenized at 3°C for 20 seconds with a Polytron ST homogenizer in enough 1.0 M NaCl, 10^{-5} phenylmethylsulfonyl fluoride to give a 10% (w/v) solution. The homogenate was shaken gently for 18 hr at 3°C and then centrifuged at 65,000 ×*g* for 60 min. The supernatant was made 5 M NaCl and the collagen was collected by centrifuging at 65,000 ×*g* for 60 min, redissolved in 0.1 M acetic acid, dialyzed against 0.1 M acetic acid and lyophilized. The tissue precipitate was extracted again with 0.1 M acetic acid and the acid soluble collagen was isolated from the extract as before. An aliquot of each sample was hydrolyzed *in vacuuo* at 100°C for 24 hr for hydroxyproline determination [11] as a measure of the collagen content.

Polyacrylamide sodium dodecyl sulfate slab gels were prepared as described by Goldberg, Epstein, and Sherr [12]. Lyophilized protein from the M NaCl extract was dissolved in sample buffer at a final protein concentration of 5 mg/ml, heated for 60 min at 55–60°C and applied to the gel. The samples were electrophoresed at 100 mA/gel until the bromphenol blue tracking dye reached the bottom of the slab. The gels were stained with Coomassie Blue. Molecular weight determinations were made using rat skin α1(I) and α2 chains and CNBr peptides of rat skin collagen for molecular weight standards as described by Furthmayr and Timpl [13].

Procollagen Peptidase Assay

Extracts from the skin of the affected and age-matched cats were assayed for procollagen peptidase activity. Substrate for the assay was the collagen extracted in 1.0 M NaCl from the affected cat skin dissolved in 0.15 M NaCl at 1 mg/ml. Enzyme was prepared from pulverized, defatted dermal tissue homogenized for 20 seconds with a Polytron ST homogenizer in 3 vol of 0.15 NaCl, 0.002 M $CaCl_2$, 0.05 M Tris-HCl (pH 7.4) at 4°C. The homogenate was centrifuged at 20,000 ×*g* for 20 min and the supernatant assayed for NH_2-terminal procollagen peptidase.

An aliquot of the tissue extract supernatant was incubated with 200 µl of the substrate preparation at 26°C for 8 or 16 hr. After incubation the collagenous proteins were separated by electrophoresis and the ratio of pNα to α chains was measured to determine procollagen peptidase activity [5].

Amino Acid Analysis

α chains from the skin of the control cat and pNα chains from the affected cat were prepared for amino acid analysis by electrophoresis on SDS-polyacrylamide gels. The 1.0 M NaCl extract was suspended in SDS-phosphate buffer at 5 mg/ml; 9 mg were applied to a 5% acrylamide slab gel and electrophoresed. Each gel was then stained with Coomassie blue for 30 min, and destained for 2 hr by diffusion. The α and pNα bands were cut from the gels and the protein was electroeluted overnight into a dialysis bag as described [14]. The purified proteins were dialyzed against distilled water, lyophilized, and then hydrolyzed

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in constant boiling 6 N HCl, 0.5% (v/v) β-mercaptoethanol, for 24 hr *in vacuuo*. The amino acid composition of each chain was determined on a Beckman 121 amino acid analyzer.

Conversion of Procollagen to Collagen by Cells in Culture

Dermal fibroblastic cells from normal and affected animals were labeled with ^{3}H-proline (25 μCi/ml) at confluent density in Dulbecco-Vogt modified Eagle medium without calf serum but supplemented with 50 μg/ml of ascorbic acid. Proteins from the culture medium were concentrated by precipitation with cold 10% trichloracetic acid and separated by electrophoresis in 5% SDS polyacrylamide slab gels [15]. Radiofluorography was performed as previously described [16].

RESULTS

Although the collagen content of affected skin was similar to that of control skin (93 μmole of hydroxyproline/gm of wet weight) the collagen in affected skin was more soluble in non-denaturing solvents (Table 1).

The soluble collagenous proteins from the affected cat contained α chains as well as significant amounts of pNα1 and pNα2 (Fig 1). These latter chains were identified by their migration during electrophoresis and by the lack of interchain disulfide bonds.

The molecular weights of pNα1 and pNα2 were calculated as 110,000 and 99,000 daltons, respectively, with α chains and CNBr peptides of the α chains as standards. The amino acid composition of these chains is consistent with the presence of considerable nontriple helical material. There is a decrease in the relative glycine and hydroxproline contents and cysteine is present where it is absent from collagen (Table II).

Procollagen peptidase activity could be demonstrated only in the extract from the normal cat skin and was not inhibited by the addition of extract from the dermatosparaxic cat skin (Table III). Extracts from normal cat skin could convert the procollagen molecules to collagen (Fig 2).

Although conversion of procollagen to collagen is limited in

TABLE I. *Solubility of collagen from the skin of normal and procollagen peptidase deficient cats*

Fraction	Hydroxyproline content (μmole HYP/gm of wet weight)	
	Normal	Procollagen peptidase deficient
1.0 M NaCl	1.0 (1%)	2.8 (3%)
0.5 M acetic acid	3.3 (3.15%)	6.9 (7.3%)
Insoluble	100.3 (95.8%)	84.1 (89.6%)

Skin was successively extracted with 1 M NaCl and 0.5 M acetic acid. The extracts and the insoluble residue were hydrolyzed and the hydrolysate was assayed for hydroxyproline as an indicator of collagen.

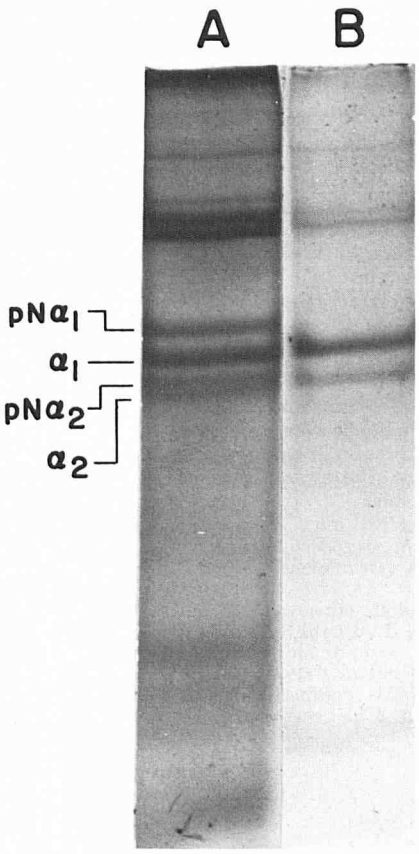

FIG 1. SDS-PAGE of collagen from the skin of a cat affected with dermatosparaxis and a control cat. Collagen extracted with 1 M NaCl, 10^{-5} M phenylmethylsulfonyl fluoride from the skin of the affected cat and a control cat were electrophoresed on SDS-polyacrylamide gels. In track A, 80 μg of collagen from the affected cat were electrophoresed and in track B, 80 μg of collagen from the control cat were electrophoresed.

TABLE II. *Amino acid composition of collagen α and pNα chains isolated and purified by SDS-PAGE from the skin of a normal and a dermatosparaxic cat*

Amino acid[a]	α1 isolated from normal cat skin	α1 isolated from dermatosparaxic cat skin	pNα1 isolated from dermatosparaxic cat skin	α2 isolated from normal cat skin	α2 isolated from dermatosparaxic cat skin	pNα2 isolated from dermatosparaxic cat skin
Aspartic acid	45	41	50	48	48	46
Hydroxyproline	100	98	93	87	88	87
Threonine	18	19	28	23	24	21
Serine	36	40	35	41	42	49
Glutamic acid	74	74	87	70	69	70
Proline	139	131	119	112	112	96
Glycine	324	333	299	332	327	305
Alanine	109	113	109	105	112	113
Valine	14	14	23	29	29	28
Half-cystine	—	—	5.0	—	—	4.0
Methionine	6.7	8.0	6.1	4.4	4.6	4.2
Isoleucine	9.3	8.2	11.	14	14	15
Leucine	20	21	21	32	32	31
Tyrosine	1.9	3.5	4.8	3.2	3.2	6.9
Phenylalanine	12.	10.2	13.	11	11	16
Hydroxylysine	4.8	6.8	9.4	8.2	8.2	22[b]
Lysine	33	35	40	23.	23	34
Histidine	2.9	4.9	3.9	7.5	7.5	6.4
Arginine	51.	46	43	50	49	46

[a] Values are expressed as residues per 1000 residues and are not corrected for hydrolytic losses. Due to the limited amount of collagen values represent the average of duplicate analysis on a single hydrolysate. Values less than 10 residues per 1000 are given to the nearest 10th of a residue.

[b] The value for the hydroxylysine in the dermatosparactic cat pNα2 is high compared with that in the cattle and sheep. The reason for this difference is not known.

TABLE III. *Conversion of pNcollagen to α1 and α2 by enzyme preparations derived from normal and procollagen peptidase deficient cat skin*

pNcollagen incubated in the presence of	pNα1 + pNα2 converted to α1 + α2
50 μl (155 μg protein) Normal skin extract	128
100 μl (311 μg protein) Normal skin extract	203
50 μl (156 μg protein) Affected skin extract	n.d.[a]
100 μl (312 μg protein) Affected skin extract	n.d.[a]
50 μl (155 μg protein) Boiled normal skin extract	n.d.[a]
50 μl (156 μg protein) Boiled affected skin extract	n.d.[a]
50 μl (156 μg protein) Affected skin extract plus 50 μl (155 μg protein) normal skin extract	111

pNcollagen was incubated as described in Materials and Methods. The collagen and pNcollagen were isolated and electrophoresed on polyacrylamide gels in sodium dodecyl sulfate buffer. After electrophoresis the protein bands were stained with Coomassie Brilliant Blue and the amount of protein in each band was determined by densitometric traces. Units are arbitrary units of integrator.

[a] Not detectable.

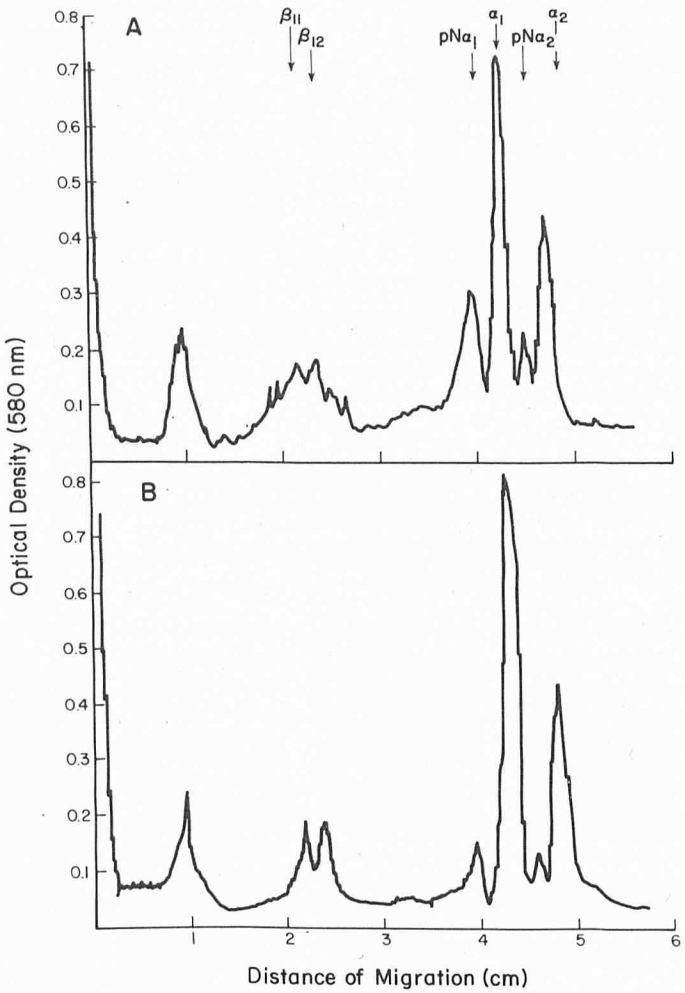

FIG 2. SDS-PAGE of pNcollagen incubated with procollagen peptidase pNcollagen from the skin of the affected cat was incubated with either (*A*) 310 μg of procollagen peptidase from the affected cat or (*B*) 310 μg of procollagen peptidase from the normal cat for 8 hr at 26°C. The collagen was isolated and electrophoresed on SDS-polyacrylamide gels. The gels were stained with Coomasie blue and scanned at 580 nm to determine the quantity of pNα chains converted to α chains. The pattern in A is identical to a control which contained no enzyme.

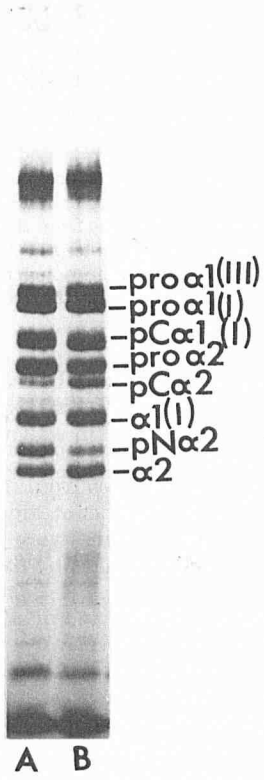

FIG 3. Radiofluorogram of ^{3}H-proline labeled collagenous proteins synthesized by dermal fibroblasts from the dermatosparactic cat (*A*) and a control (*B*). Although conversion to collagen is incomplete even in the normal, the affected cat accumulates considerably more pNα2 than control. Because pNαl co-migrates with proα2 in this system, the accumulation of that precursor is not demonstrable.

cell culture there was an accumulation of pNα chains in medium of dermatosparaxic cells (Fig 3).

DISCUSSION

Failure to convert procollagen to collagen could be a result of defective conversion enzymes [4,6] or of abnormalities in the substrate [17]. In the animal described in this report the evidence points to a defect in the NH_2-terminal procollagen peptidase since both pNα1 and pNα2 chains accumulate in dermis and the enzymatic activity could not be readily demonstrated in the affected skin.

In contrast to the dermatosparactic sheep in which the enzyme defect is severe and the survival is brief, the cat has survived longer than 2 yr. Furthermore, as judged by the relatively small amounts of pNα1 and pNα2 in skin, the enzyme defect appears to be incomplete. Despite this it was not possible to detect NH_2-terminal peptidase activity from affected skin. Several explanations could account for this finding, including tight binding of the abnormal enzyme to the substrate which is not disrupted by mild solvents. The relatively mild disease in this animal is reflected in the ultrastructural findings as well [18].

Evidence from several laboratories now suggests that the NH_2-terminal procollagen extensions are cleaved first in tissues and is quickly followed by cleavage at the COOH-terminus [9,19]. The COOH-terminal fragment appears to have considerably more bulk than the NH_2-terminal extension [8,20] and it may be that abnormalities in the COOH-terminal conversion process are lethal since molecular aggregation, fibril formation, cross-link formation and bundle aggregation may be so disrupted that normal tissue development cannot occur. The accumulation of only pNα1 and pNα2 chains in this animal and in others with dermatosparaxis again suggests the distinction between the terminal peptidases recently confirmed on the basis of differential activities [10] of isolated proteins.

Although conversion of procollagen to collagen by cells in culture is limited even in normals, the abnormality can be detected in cell cultures from this affected animal because they accumulate pNα chains. Since some human diseases of collagen metabolism are amenable to study in cell culture [21,22] it is likely that similar approaches to animal disorders will be profitable.

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Announcement

In 1980, the American Board of Dermatology will give its certifying examination in Chicago, Illinois, on November 2 and 3; the deadline for receipt of applications is April 1, 1980.

The Dermatopathology special competence examination will be held in Miami Beach, Florida, on November 13, 1980; the deadline for receipt of applications is July 1, 1980.

Also, in 1980, the Board will offer the annual In-Training examination to all residents-in-training and interested practicing dermatologists in the spring. Complete information will be mailed to all training directors in late February or early March, 1980.

For further information on any of these examinations, please contact: Clarence S. Livingood, M.D., Executive Director, American Board of Dermatology, Henry Ford Hospital, Detroit, MI 48202.