

BIOSYNTHESIS OF ELASTIN IN PSEUDOXANTHOMA ELASTICUM

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Abstract. Elastin biosynthesis was studied on biopsy specimens from lesions of the axillary region and from clinically uninvolved skin of the sacral region of 8 patients with pseudoxanthoma elasticum (PXE), as well as from corresponding areas of 6 control subjects. A significant increase of (^{14}C)glutamine and of total ^{14}C (proline + hydroxyproline) was found in PXE skin lesions, as compared with corresponding areas of controls. The increase of (^{14}C)glutamine was significantly greater than that of (^{14}C)proline. Clinically uninvolved skin of PXE patients showed no abnormalities in respect of the parameters studied. The results suggest an increased biosynthesis of an abnormal elastin with elevated glutamine content in the PXE lesions.

Because of the morphological finding of calcification of elastic fibres as the first visible abnormality in pseudoxanthoma elasticum (PXE) (4, 5, 9, 11) it was decided to study the biosynthesis of elastin which might involve biochemical abnormalities invisible in the electron microscope. Previous biochemical studies have shown a significant increase of calcium in skin lesions of PXE (2, 7, 16). Elastin and glycosaminoglycans have also been reported to be increased (16, 17). Smith et al. (16) and Rodnan & Yogodnik (15) found no change in the content of hydroxyproline in dermal elastin of PXE, while Avogadro & Castellani (1) found a decreased hydroxyproline content within the total amino acid fraction of dermal elastin. Amino acid analysis of dermal elastin in PXE (1, 15) has shown an increased concentration of glutamic acid and aspartic acid. We decided to study the incorporation of (^{14}C)glutamine in PXE elastin, because a pilot study of ours

showed a higher uptake of (^{14}C)glutamine than of (^{14}C)glutamic acid in elastin of normal skin. Normally, glutamine is known to be freely converted into glutamic acid (6, 18) which is one source of proline and hydroxyproline (12).

MATERIAL AND METHODS

Chemicals

Uniformly labeled (^{14}C)L-lysine 223 $\mu\text{Ci}/\mu\text{mole}$, (^{14}C)L-proline 180 $\mu\text{Ci}/\mu\text{mole}$, and (^{14}C)L-glutamine 150-200 $\mu\text{Ci}/\mu\text{mole}$, were purchased from New England Nuclear, Boston.

Papain. E. Merck A. G., Darmstadt.

Collagenase. Worthington Biochemical Corp. (CLSPA).

N-hydroxyethylpiperazine-N-2-ethanesulfonic acid buffer. (HEPES) Calbiochem.

Ampicillin. (Pentrexyl®—Lundbeck, Copenhagen, Denmark).

Skin samples

The tissues studied were skin biopsies of PXE patients and normal controls matched for age, sex and area. Six axillary and 8 sacral biopsies from 8 PXE patients and 5 axillary and 6 sacral specimens from 6 controls were taken on one day and by the same person.

Preparation of samples. The samples were divided into 3 portions. Part 1 was used for the determination of dry weight. Part 2 was incubated with 5 μCi of (^{14}C)Pr. Part 3 was incubated with 5 μCi of (^{14}C)Glut. Dry weight was determined as indicated in the accompanying paper (3).

Samples were pre-incubated at 37°C for 1 hour and then incubated in the presence of the corresponding labelled amino acid for 24 hours. To end the incubation, samples were cooled rapidly. The samples were submitted to the procedure indicated in the accompanying paper (3) i.e. papain digestion followed by collagenase digestion. The undigested material separated as a pellet by centrifugation was treated with 0.1 N NaOH at 100°C for 45 min (8). The material was then cooled and centrifuged in a Servall centrifuge at 13 000 g at 4°C. The supernate alkali-soluble collagenase-undigested material was discarded and the pellet alkali-insoluble collagenase-undigested material (elastin) was analysed.

Abbreviations

Lys = lysine

Pr = proline

Glut = glutamine

Hypro = hydroxyproline

Table I. *Hydroxyproline content in elastin extracted from skin of PXE patients and normal controls*

Diagnosis	No.	Skin region	μg Hypro per 100 mg wet weight of skin
PXE	6	Axillary	21.79 ± 8
	8	Sacral	5.14 ± 1
Control	5	Axillary	8.12 ± 3
	6	Sacral	4.77 ± 2

Hydrolysis was performed by treatment of the pellet with 6 N HCl for 72 hours at 110°C . After hydrolysis, HCl was evaporated under vacuum at 65°C . The evaporated samples were dissolved in distilled water. Total hydroxyproline was determined on an aliquot according to Kivirikko et al. (13). The total $^{14}\text{C}(\text{Pr} + \text{Hypro})$ and $^{14}\text{C}\text{Glut}$ content in an aliquot of the corresponding portions labelled with $^{14}\text{C}\text{Pr}$ and $^{14}\text{C}\text{Glut}$ were counted in a liquid scintillation counter. Results are expressed as means $\pm$ S.E.

Statistical analysis: Student's *t*-test was used to determine the significance of the differences observed. The Mann-Whitney U-test was used to evaluate age differences in the data collected.

RESULTS

Total hydroxyproline. No significant disease differences were found in the hydroxyproline content in elastin extracted from part 3, although there was a tendency for the hydroxyproline of the PXE lesions to be higher than the corresponding areas of the controls. Two out of 6 patients showed a very marked increase in the content of hydroxyproline, while the other 4 values were about normal. No significant area differences were observed in the controls (Table I).

Total ^{14}C (proline + hydroxyproline). Significant differences between axillary areas were observed when the total $^{14}\text{C}(\text{Pr} + \text{Hypro})$ of elastin of both groups was studied ($P < 0.02$), the values being higher in the lesions. No significant differences were observed between the sacral areas of the two groups (Table II). No statistically sig-

nificant differences were observed in the $^{14}\text{C}(\text{Pr} + \text{Hypro})$ of elastin between axillary and sacral areas of controls.

No significant age difference was found when comparing the $^{14}\text{C}(\text{Pr} + \text{Hypro})$ content in sacral elastin from 2 subjects below 30 years, with 6 subjects over 50 years of age.

$^{14}\text{C}\text{glutamine}$. There was an increased $^{14}\text{C}\text{Glut}$ content in elastin from the axillary area of PXE patients when compared with the corresponding area of controls. The differences were statistically significant ($P < 0.001$). Elastin extracted from the sacral areas of both groups showed no differences. No significant differences in the content of $^{14}\text{C}\text{Glut}$ were observed when comparing the axillary and sacral areas of the controls (Table II). On comparison of the $^{14}\text{C}\text{Glut}$ content in elastin from the control sacral areas in 2 subjects below 30 years, with 6 subjects over 50 years of age, no significant age differences were observed.

Ratio $^{14}\text{C}\text{glutamine}$ to $^{14}\text{C}\text{proline}$. The differences between the ratios $^{14}\text{C}\text{Glut}$ to $^{14}\text{C}\text{Pr}$ of the axillary area of patients and controls was significant at the 5% level (Table II). The corresponding ratios of the sacral areas were not significantly different. No area differences in the controls were noticed.

DISCUSSION

A higher uptake of $^{14}\text{C}\text{Glut}$ than of $^{14}\text{C}\text{Pr}$ by newly synthesized elastin of PXE lesions suggests a localized abnormality in the incorporation of $^{14}\text{C}\text{Glut}$ in PXE, while an increased content of both $^{14}\text{C}\text{Glut}$ and $^{14}\text{C}(\text{Pr} + \text{Hypro})$ in elastin of the lesions suggests an increased elastin biosynthesis in PXE. In an abstract, Rodnan & Yogodnik (15) reported a significant increase in the aspartic and glutamic acid components of elastin in two PXE patients when compared with two

Table II. *Changes in $^{14}\text{C}\text{Glut}$ and $^{14}\text{C}(\text{Pr} + \text{Hypro})$ content in elastin extracted from skin of PXE patients and normal controls*

Diagnosis	No.	Skin region	^{14}C Glut dpm per 100 mg wet weight of skin	^{14}C (Pr + Hypro) dpm per 100 mg wet weight of skin	$\frac{^{14}\text{C} \text{ Glut}}{^{14}\text{C} \text{ Pr}}$
PXE	6	Axillary	$3\,748 \pm 500$	$10\,027 \pm 1\,796$	0.37
	8	Sacral	180 ± 31	$1\,939 \pm 529$	0.092
Control	5	Axillary	665 ± 90	$3\,791 \pm 1\,116$	0.17
	6	Sacral	346 ± 147	$1\,820 \pm 468$	0.19

control subjects. Our finding of increased incorporation of (^{14}C)Glut in elastin of PXE lesions is in agreement with the above-mentioned report (15) if normal metabolic conversion of glutamine into glutamic acid is considered. Among other changes with ageing and arteriosclerosis, an increase of glutamic and aspartic acids and their amides in aortic elastin has been reported (8). Carboxylate anions in combination with threonine hydroxyls (10, 14) have been implicated in initiating calcification processes in the arterial wall. Increased calcium with no parallel increase of phosphorus has been found in one out of five PXE patients (2). It is evident that at least in that patient, calcium did not occur as calcium phosphate.

We found no evidence that our finding of an increased content of (^{14}C)Glut in PXE elastin represents an ageing phenomenon, as has been suggested for aortic elastin (8).

Our findings of unchanged amounts of elastin as expressed as hydroxyproline in PXE lesions of 4 patients are in agreement with the report of Rodnan & Yogodnik (15), while the increased amount of elastin in 2 patients is in agreement with Smith et al. (16). However, it does not appear in these reports whether corresponding areas were compared. There seems to be an increased build-up of elastin in the lesions of PXE patients. The absence of biochemical alterations in the sacral region of PXE patients reported in this and the previous paper (3) accords with the absence of ultrastructural alterations in the gluteal region of five PXE patients (5) and normal calcium content in the sacral area of six PXE patients (2). Thus, despite the widespread nature of PXE, the skin alterations appear to be restricted to certain areas, particularly the flexural areas.

Comparing the abnormalities found in elastin and collagen (3) in the PXE lesions, it appears that a change in glutamine is not an abnormality which the two macromolecules have in common.

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