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Biosynthesis of Insoluble Elastin in Hamster Lungs during Elastase-Emphysema (40055)

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Recently, an experimental model of emphysema induced by elastase has been extensively studied (1–4). In the elastase model of experimental emphysema, a single injection of elastase caused diffuse destruction of lung structural components and led to a lung morphology which resembled human panlobular emphysema. It has been observed in this model that the elastin content of the lung decreased shortly after the elastase instillation but gradually returned to a nearly normal value within 60 days (5). The possibility of the replacement of this connective tissue fiber after the elastase-injury by active synthesis of elastin has been indicated (5). This report describes further studies of elastin synthesis in the lungs of hamsters with elastase-induced emphysema.

Mature elastin has been considered a biologically stable and metabolically inert substance (6). For example, in aortic tissues of mature rats, Slack (7), Kao *et al.* (8) and Walford *et al.* (9) showed that elastin has no active turnover. Previous work in our laboratory has shown that elastin in lung tissue is also inert, with little or no synthetic activity of the protein in the mature lungs of normal hamsters (Yu, S.Y. and Keller, N.R., unpublished data). Therefore, in mature hamsters, it is difficult to label *in vivo* with a radioactive precursor by the conventional technique for studying the metabolism of elastin.

The following three procedures were used for the studies of elastin synthesis.

(a) The synthetic activity of lung elastin was measured by comparing rates of incorporation of [^{14}C]-L-proline into elastin between the experimental and control lungs. The [^{14}C]-L-proline was administered by a series of injections which followed the elastase-instillation. (b) The synthetic activity of lung elastin was also studied in hamsters which had been labeled with a series of injections of [^{3}H]-L-proline during a rapid growth period and subsequently injected with elastase 3 months after the labeling. (c) Finally, since desmosines in elastin are characteristic amino acids and only found in fully cross-linked elastin, the rate of incorporation of [^{14}C]-L-lysine into desmosines found in lung extracts from the experimental and control group was compared.

Materials and methods. Incorporation of [^{14}C]-L-proline into lung elastin. All hamsters used in the experiments were obtained from Engle Laboratory Inc., Farmersberg, IN.

Experimental emphysema was developed in mature male hamsters, weighing 115–120 g each, by an intratracheal instillation of 0.3 ml of saline solution containing 0.25 mg or 25 Sachar's units of porcine pancreatic elastase (2). For controls, the animals were instilled with 0.3 ml of saline solution. Three days after the instillation of elastase, all hamsters, 25 controls and 21 experimentals, received a series of 14 intraperitoneal injections of [^{14}C]-L-proline, at intervals of 12 hrs; each dose containing 0.2 ml of 0.9% NaCl solution and 4 μCi of uniformly labeled [^{14}C]-L-proline, SpA 225 Ci per mole (New England Nuclear).

Preparation of insoluble elastin. Lungs were pooled respectively, homogenized with 100 vol of cold 0.9% NaCl, and then the insoluble residues were extracted in polyethylene plastic centrifuge tubes successively with the following solutions: 0.16 *M* NaCl solution at 4°, 5×; 5 *M* of guanidine · HCl, 0.1 *M* Tris pH 7.4, at 27°, 2×; 5 *M* of guanidine · HCl, 0.1 *M* Tris, 50 m*M* dithiothreitol and 0.1 *M* EDTA pH 7.4, at 27°, 2× (10); water, 3×; autoclaving at 15 lb for 4 hr, 2×; acetone, 3×; and ether, 2×. The insoluble residue was treated with 0.1 *M* NaOH at 98° for 45 min.

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The extracted residue (elastin) was washed several times with water, finally resuspended in water, lyophilized, and weighed. Dry elastin, 3–5 mg, was weighed on a filter paper and combusted in a Packard's Sample Oxidizer Model 306. Radioactivity was counted with a liquid scintillation counter, and expressed as specific activity.

Prelabelling Hamsters with [^{3}H]-L-Proline. Young male hamsters, litter mates, initially weighing 54 ± 5 g each, were chronically labelled with a series of intraperitoneal injections, one dose daily of 0.1 ml saline containing 2.8 μCi of [^{3}H]-3,4-L-proline (33.5 Ci/mmole, New England Nuclear), and the injections were repeated 22×. By the end of the labelling the animals weighed 88 ± 7 g and then were kept until body weight reached 117 g, which was approximately 3 months after the labelling. The solutions of elastase or saline were intratracheally instilled in the prelabelled hamsters, and lung elastin was prepared and radioactivity was measured as described previously (see above).

Incorporation of [^{14}C]-L-lysine into desmosines. Mature male hamsters, 18 of the elastase-instilled and 11 controls, were used. Three days after the elastase-instillation each animal received a series of 30 intraperitoneal injections, at 12-hr intervals, each dose containing 0.2 ml of saline and 1.1 μCi of uniformly labeled [^{14}C]-L-lysine, 279 mCi/mmole (New England Nuclear). The animals were sacrificed 18 days after the elastase-instillation. Lungs were pooled into control and experimental groups and extracted as described previously (see above), and the "autoclave" residue was obtained. The "autoclave" residue was hydrolyzed with 6 *N* HCl, and desmosines isolated. Desmosines in the hydrolyzate were separated by a preparative column of Aminex A-5 resin, 1.5 × 15.5 cm (Bio-Rad Lab), at 50°, by eluting with 0.15 *M* pH 4.5 pyridine–acetate buffer and 0.3 *M* pyridine (11). The corresponding fractions of isodesmosine and desmosine were collected, pooled, and the solvents evaporated. Each compound was further separated by an analytical column 0.9 × 13 cm with Beckman PA-35 resin and eluted at a flow rate of 68 ml/hr with sodium citrate buffers under the following conditions; at 55°, 0.2 *N*, pH 4.35 for 30 min, 0.2 *N*, pH 4.50 for 40 min and 0.38 *N*, pH 4.50 for 60 min. The fractions were collected, and aliquots of the appropriate fractions were used for determination of the amount of desmosines in the analytical column. A second aliquot was used for counting radioactivity by a liquid scintillation counter.

The elastase-instillation produced a distension of normal lung structure, which has been studied using the mean linear intercept method to quantify this dilation (2) (12). The present study yielded a similar change in the gross morphology of lungs of the elastase-instilled hamsters.

Results. Post-elastase labelling. Elastin synthesis, studied by the incorporation of [^{14}C]-L-proline in which the isotope was administered after the elastase-instillation, showed that the specific activity of elastin in the lungs of the elastase-instilled groups increased 8–14 fold over those of the respective control (Table I). Within the experimental group, there was a reduction of the specific activity in lung elastin of day 30 compared to that of day 18 which presumably was a result of the dilution of the radioactive elastin by nonradioactive elastin synthesized during the period of days 18 and 30. These data suggested that elastin synthesis was active, not only in the period 3 to 18 days after the elastase-instillation but also during the period 18 to 30 days.

Preelastase labelling. Elastin synthesis, studied in the prelabelled hamsters, showed that the specific activities of lung elastin were lower in the elastase-instilled groups as compared to those of controls (Table II). Since

TABLE 1. INCORPORATION OF [^{14}C]-PROLINE INTO LUNG ELASTIN OF CONTROL AND ELASTASE-INSTILLED HAMSTERS.[a]

	Sp Act [^{14}C] dpm/mg	Average	Rate of incorporation
Control day 18	165[b]		1
Control day 30	208[b]	$\bar{x}$ = 187	1
Elastase day 18	2552[b]		14
Elastase day 30	1429[b]		8

[a] Hamsters were intratracheally instilled with the elastase, and then a series of [^{14}C]-L-proline injections was carried out. Control groups at days 18 and 30 contained 15 and 10 hamsters respectively, while the elastase-instilled groups contained 11 and 10 hamsters.

[b] Each experiment was based on at least two determinations of the radioactivity, and the average was obtained.

TABLE II. SPECIFIC ACTIVITIES OF LUNG ELASTIN FROM PRELABELED HAMSTERS, CONTROL AND ELASTASE-INSTILLED.[a]

	Sp Act ^{3}H dpm/mg	P^c	% decrease[b]
Control day 18	640 ± 48[b]		
Control day 30	662 ± 50[b]		
Elastase day 18	561 ± 62[b]	NS	14%
Elastase day 30	429 ± 62[b]	$P < 0.05$	34%

[a] Hamsters were prelabeled with [^{3}H]-L-proline during a rapid growth period and then 3 months after the labeling instilled with the elastase intratracheally. The animals were sacrificed 18 and 30 days after the elastase, with the control groups containing five animals and the experimental groups containing four animals.

[b] Mean ± SEM. The data were compiled from three experiments.

[c] Compared with the corresponding control; NS, not significant.

[d] Compared with the average of control day 18 and 30.

mature-normal lung showed no active synthesis of elastin, a decrease in the specific activity of the prelabelled elastin by incorporation of non-radioactive elastin after the elastase-instillation indicated the synthetic activity of elastin. This decrease in the specific activity after the elastase-instillation was observed in lung elastin of day 30 ($P < 0.05$).

[^{14}C]-L-lysine incorporation into desmosines. Although the insoluble elastin as prepared showed the characteristic amino acid composition with no evidence of possible contamination by other impurities (5), this method is often subjected to criticism since the elastin prepared is the insoluble residue after the exhaustive extractions which include hot 0.1 *N* NaOH. Thus the possibility of losing elastin by this treatment or of contamination by other insoluble materials might remain, especially in the case of extracting tissues of diseased, aged or extremely young animals. However, studies using desmosines, which are amino acids exclusively found in mature-insoluble elastin, should nevertheless provide an insight into the metabolism of insoluble elastin. The result of the incorporation of [^{14}C]-L-lysine into desmosines is presented in Table III. The specific activities of desmosines isolated from the lung fractions obtained from the elastase-instilled hamsters were higher than those of the corresponding controls, 3600 and 3800 dpm per μmole of isodesmosine and desmosine, vs 309 and 190 dpm of isodesmosine and desmosine, for the experimentals and control, respectively.

Discussion. In lungs of the hamsters with elastase-induced emphysema, there was a decrease in the amount of elastin shortly after the injection of elastase, presumably resulting from proteolysis by the enzyme (Table IV); thereafter, the amount of elastin per lung rose and then became stable within 18 days after the elastase-injection. In addition to this active elastin synthesis in lungs of the elastase-instilled hamsters, the accelerated turnover of elastin persisted beyond the point of the restoration of nearly-normal amounts of elastin.

This activation of elastin synthesis after lung injury by elastase was accompanied by simultaneous collagen synthesis (13), therefore we believe that this activation of mesenchymal tissue resulted from a tissue response to the specific injury to the lung. The synthesis of elastin which was observed in hamster lungs after the elastase-instillation is

TABLE III. INCORPORATION OF [^{14}C]-L-LYSINE INTO DESMOSINES IN AUTOCLAVE RESIDUES OF HAMSTER LUNGS, CONTROL AND ELASTASE-INSTILLED.[a]

	No. of lungs	Isodesmosine[b] dpm/μmole	Desmosine[b] dpm/μmole
Control	11	309	190
Elastase	18	3808	3636

[a] Hamsters were intratracheally instilled with the elastase solution and then a series of [^{14}C]-L-lysine injections was carried out. The animals were sacrificed 18 days after the elastase-instillation, and then the lungs were pooled. The autoclave residues were prepared after a series of extractions with solutions of NaCl, guanidine · HCl and autoclaving.

[b] Desmosines were obtained from hydrolysates of autoclave residues of hamster lungs after purification on ion exchange columns. The amount of desmosines were estimated by the ninhydrin reaction using lysine as the standard.

TABLE IV. ELASTIN AND TOTAL DESMOSINES IN HAMSTER LUNGS OF CONTROL AND ELASTASE-INSTILLED.

	Days after Injection[a]	Desmosines μmole/lung	Elastin[b] mg/lung
Control	3 (2)	0.251	2.1
Elastase	3 (2)	0.092	0.8
	18 (2)	0.221	1.8
	30 (2)	0.245	1.9

[a] Numbers in parentheses indicate number of animals used.

[b] The estimation was based on the concentrations of 0.070 μmole desmosine and 0.055 μmole isodesmosine per mg elastin.

seemingly a reparative process in response to the elastase-injury.

Kuhn and Tavassoli (14) observed from morphological evidence that the rearrangement of the lung structure had been continuing up to 2 months after the elastase-instillation in similar experiments. This suggests the possibility that the synthesis and degradation of elastin might be activated and persisted for a long time in these lungs. There is some evidence from our data (Table II) which suggested that the synthetic activity in lungs remained elevated even 18 days after the elastase-instillation, although it is not certain whether the elastin synthesis continues beyond one month after the elastase-instillation. If the synthesis of elastin were continuing in lungs of the hamsters with elastase-induced emphysema, the degradation of elastin might have occurred to balance out the increase.

The insoluble elastin, prepared by the present method, probably was "pure" lung elastin and contained little or no contaminating substances such as tropoelastin (15) or microfibril (10). The presence of the latter compound was negligible because the elastin prepared contained no significant amount of polar amino acids. However, despite the anatomically distinguishable appearance of lung elastic fibers in the elastase-instilled hamsters (5) (12), our studies failed to show any significant change in the amino acid composition of lung elastin obtained from hamsters 4 months after the elastase-instillation. This might be due to the hot alkaline treatment *per se* since Sandberg pointed out that elastin prepared from different tissues and species by this method showed similar amino acid composition because the hydrolytic nature of the reagent reduced elastic fiber to a common composition (16). Therefore, the possibility of the presence of a different elastic tissue in the emphysematous lungs might still remain.

In the lung, elastin occurs in airways, vascular trees, alveolar septa, and pleura. It is, however, impossible to identify the exact site of the synthesis by the present method, despite the demonstration of elastin synthesis in lungs of the hamsters with elastase-induced emphysema. By morphological observations (12) (17), the site of the elastase-injury was observed more frequently in perivascular areas, alveolar septa, and pleura; however, discrete and fibrillar elastic fibers were often found in alveolous septa, and a spongelike elastic structure was seen in peripheral areas of the elastic lamella under the mesothelial plane. Although these elastic fibers were observed only in lungs of hamsters with the elastase-induced emphysema, it is not known whether these fibers are some of the degradated products resulting from the elastase-instillation or newly synthesized fibers.

Following an injury, the normal tissue response is to repair by synthesizing collagenous fibers but not elastic fibers. The reason for the synthesis of both elastic and collagenous fibers in the emphysematous lungs is not known, although a similar situation was also observed in cultured aortic cells which formed both collagen and elastin (18–20).

Summary. The synthesis of elastin *in vivo* in normal-mature animals is difficult to demonstrate because of a very slow turnover of this protein. Thus most of the studies of elastin synthesis have been limited to aortic tissue cultures or in *in vivo* young animals. Our data indicate that the lung, upon elastase-injury, synthesized elastin at demonstratively faster rates than normal. However, the mechanism by which the activation of elastin synthesis occurs in the emphysematous lungs remains for further study.

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