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Research Article

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Urinary Excretion of Elastin Peptides Containing Desmosine after Intratracheal Injection of Elastase in Hamsters

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ABSTRACT The intratracheal injection of pancreatic elastase results in an acute loss of elastin from the lungs of hamsters and the development of emphysema. We used measurements of the unique covalent cross linking amino acids of elastin, desmosine and isodesmosine, to quantitate elastin. Direct measurements on the lungs estimated an average loss of elastin of 57% after elastase injection. Elastin breakdown products were also quantitated in the urine and feces after injection. An average of 8.8 nmol of desmosines was recovered from the urine of each hamster. This amount represented the desmosines from 61% of the elastin lost from the lungs. Desmosine and isodesmosine existed in the urine in peptide fractions that ranged from 9 to 27,000 daltons with an average of 13,000. Only trace quantities of desmosines could be detected in feces. Desmosines injected intraperitoneally were completely recovered in the urine, and radioactive tracer studies failed to reveal in vivo catabolism of injected desmosines. These results suggest that measurement of urinary desmosines holds promise for the study of elastin turnover.

INTRODUCTION

Pulmonary emphysema can be produced in animals by the intratracheal injection of papain (1), or elastase (2), but apparently not with collagenase (3, 4). These findings suggest that elastolytic activity is responsible for the production of emphysema. In support of this theory, the elastic fibers of the lung are found disordered or lost within hours after the intratracheal injection of elastase (5), even though the morphologic changes of emphysema are inconspicuous until several days later. Quantitative studies indicate an acute reduction in the elastin content of the lung followed by a gradual return to normal levels over a period of several weeks (6).

The fate of the elastin lost from the lungs in this experimental model has not been studied previously. We expected the breakdown products of elastin to be excreted in the urine or the feces and designed this study to determine whether these excretion products could be measured. Elastin levels were determined from measurements of the unique cross linking amino acids, desmosine and isodesmosine. The results revealed that elastin peptides containing desmosine can be recovered in the urine after the intratracheal injection of elastase. Additional studies failed to demonstrate catabolism of desmosines in vivo.

METHODS

Elastase injection. Three groups of three hamsters were studied in two separate experiments. Each hamster weighed 140–150 g and anesthesia was given with intraperitoneal Surital (Parke, Davis, Inc., Detroit, Mich.). With the animal in the supine position, a small incision was made in the neck, and the trachea was exposed. After passing a needle between tracheal rings, 20 U of porcine pancreatic elastase (Elastin Products, St. Louis, Mo.) dissolved in 0.2 ml of 0.15 M NaCl were injected in a caudal direction. The injected liquid was distributed in the lungs by rotating the hamster for ≅1 min. The wound was then sutured. Control animals were uninjected.

Collection of excreta. Immediately after injection, each group of hamsters was placed in a metabolic cage for 4 days for collection of urine and feces. Feces were collected on a screen under the cage bottom while the urine passed into a beaker further below. While in this apparatus, the animals received only water and food that was monitored, free of elastin. After removal of the feces, the cage and screen were rinsed with water into the beaker used for collection of the urine.

Measurement of elastin peptides. Pooled urine was dialyzed in a 3,500-mol wt dialysis sac and concentrated to dryness by flash evaporation. The concentrate was hydrolyzed for 72 h in 6 N HCl at 100°C. The feces were homogenized and similarly hydrolyzed. Quantitation of desmosine and iso-

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desmosine was performed according to a technique recently described (7). The acid hydrolysates of urine and feces were concentrated to dryness, redissolved in small quantities of water, streaked onto sheets of Whatman 3MM paper (Whatman, Inc., Clifton, N. J.), and dried. The sheets were then placed in a developing chamber (Scientific Manufacturing Industries Chromatocab, Emeryville, Calif.), and chromatographed for 72 h with a mixture of butanol, acetic acid, and water (4:1:1). Desmosine and isodesmosine, which are insoluble in this medium ($R_E = 0$) (8), remained at the origin and were thereby virtually isolated. After drying the paper, strips were cut at the origin and eluted with water in a closed chamber until 1.5 ml had been collected. This eluate was placed on a Beckman 116 amino acid analyzer (Beckman Instruments, Inc., Fullerton, Calif.) programmed especially for quantitation of desmosine and isodesmosine (7). From amino acid analyses of purified normal hamster lung elastin in other experiments, we found ≅20 nmol of desmosine and isodesmosine per mg of lung elastin. With this value it was possible to calculate the total lung elastin from which the desmosines had been derived after elastase injection.

Characterization of urinary elastin peptides. Unhydrolyzed urine from elastase-injected hamsters was passed through a gel filtration column of Sephadex G-75 (Pharmacia Fine Chemicals, Piscataway, N. J.) and eluted with 0.01 M $NaHPO_4$ buffer, pH 7.4, 0.15 M in NaCl. Optical density measurements were made on the eluate at 280 nm.

Determination of lung elastin loss. In a series of three preliminary experiments, groups of four hamsters were injected intratracheally with elastase and sacrificed 1, 3, 24, and 72 h after injection. The lungs were removed and perfused via the pulmonary artery with Triton X-100 (Rohm and Haas, Co., Philadelphia, Pa.) solution 0.1% with disodium EDTA 0.1% to remove as much blood as possible. Further removal of blood was accomplished by dicing the lungs and washing for 12 h in alternate baths of 0.15 M NaCl solution and distilled water. Subsequently, the lungs were hydrolyzed in acid and analyzed for desmosines in the manner already described.

In the desmosine recovery studies, 3 h was chosen as the optimum time after elastase injection for lung analysis, because we have found that lung elastin consistently reaches a nadir at this time. The loss of elastin was, therefore, estimated from measurements of the differences between the lungs of the uninjected and the 3-h postinjection animals.

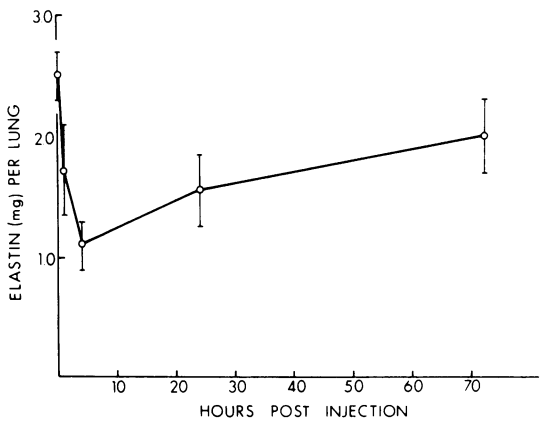

FIGURE 1 Elastin content of hamster lungs 1, 3, 24, and 72 h after elastase injection. Each point is the average of 12 hamsters with SD.

TABLE I
*Desmosine Content of the Urine from Hamsters Receiving Intratracheal Elastase**

	Des + Ides/hamster	Elastin/hamster‡
	nm	*μg*
Experiment A		
Control	0	0
Group 1	7.9	395
Group 2	11.1	560
Group 3	10.5	525
Experiment B		
Control	0	0
Group 1	7.4	370
Group 2	7.0	350
Group 3	8.9	445

* Values for each group represent the pool of 3-day urine collections for three hamsters in each group.
‡ Elastin was calculated from the desmosines with the value of 20 nM desmosines/mg elastin.

These hamsters weighed the same and had been injected at the same time as the test hamsters used for urine collection. A matched group of three uninjected hamsters was also studied.

Urinary recovery of intraperitoneal desmosines. Three 100-g hamsters each received an intraperitoneal injection of a solution containing 5.6 μg desmosine and 5.4 μg isodesmosine. Urine was collected over the subsequent three days and analyzed as already described.

A separate 100-g hamster was injected intraperitoneally with 50 μl of a solution containing [^{14}C]desmosine and [^{14}C]-isodesmosine. These labeled amino acids had been produced in short-term tissue culture of fetal calf ligament with uniformly labeled [^{14}C]lysine added to the medium. The hydrolyzed urine sample from the injected animal was placed on the amino acid analyzer and the eluate was collected in 2-min fractions. Fractions of the radioactive standard solutions were similarly collected for comparison. Radioactivity of each fraction was quantitated by liquid scintillation spectrometry.

RESULTS

Estimation of lung elastin loss. Elastin was removed from the lungs very rapidly following elastase injection, reaching a nadir at ≅3–4 h (Fig. 1). Resynthesis of elastin was evident within 24 h, and by 72 h 50% of the elastase-digested elastin had been replaced.

Urinary recovery of elastin peptides. Desmosines were not detectable in the urine of the uninjected control group of hamsters. In contrast, the urine of the 18 elastase-injected hamsters (six groups of three hamsters in each) contained an average of 8.8±1.5 nm of desmosine (Des) and isodesmosine (Ides) per hamster over the three days after injection (Table I). Of note, better than 95% of all the desmosines recovered appeared in the first 2 days after injection. Only trace amounts of desmosine and isodesmosine were found

in fecal hydrolysates and probably represent urinary contamination.

When these urinary levels were related to the loss of lung elastin measured directly (1.09 mg), a fractional recovery in the urine was determined. In separate experiments, we found that desmosines added directly to normal urine were incompletely recovered. Absolute losses were small, but when 10 μg of desmosines were added, the recovery ranged from 72–93%. A desmosine standard added to urine was carried with each experiment so that we could compare the amount of desmosines recovered in the urine to the calculated maximum loss of elastin from the lungs of weight-matched hamsters 3 h after elastase injection. The overall average elastin recovery of the two experiments was 61%.

The molecular weight of urinary elastin peptides was estimated by passing unhydrolyzed urine through a gel filtration column of Sephadex G-75 (Pharmacia Fine Chemicals) (Fig. 2). OD measurements of the eluate at 280 nm revealed two distinct peaks. Desmosine and isodesmosine were recovered only from the second peak that contained the lower molecular weight peptides; this peak ranged from 9 to 27,000 daltons with maximal absorption at 13,000 daltons.

Urinary recovery of intraperitoneal desmosines. After intraperitoneal injection of 5.6 μg desmosine and 5.4 μg isodesmosine into each of three hamsters, pooled urine collected over the subsequent 3 days was found to contain an average of 98% of the injected amino acids. This result suggests that catabolism of the desmosines was negligible in vivo.

Confirmatory evidence was obtained after intraperitoneal injection of a solution that contained [^{14}C]desmosine and [^{14}C]isodesmosine (Fig. 3). Scintillation counting of fractions separated on the amino acid analyzer column revealed superimposition of the radioactive peaks from both hydrolyzed urine and standard solutions. New peaks did not appear, so that metabolic alteration of the cross linking amino acids was not detected.

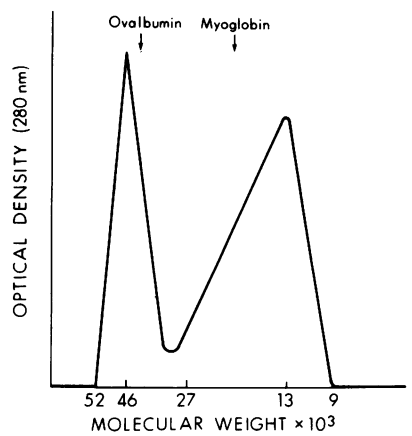

FIGURE 2 Gel filtration of urine from elastase-injected hamster on Sephadex®-75 (Pharmacia Fine Chemicals). OD measurements were made at 280 nm. Desmosines were present only in the peak ranging in mol wt from 9,000 to 27,000 daltons.

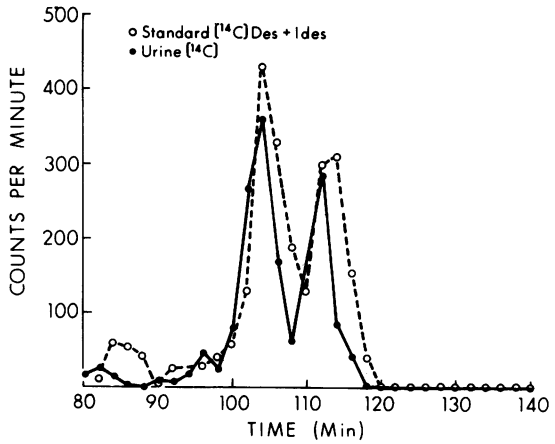

FIGURE 3 Radioactivity of standard solution of [^{14}C]desmosine (Des) and [^{14}C]isodesmosine (Ides) and of hamster urine after intraperitoneal injection of the solution. Both the solution and the urine were collected in 2-min fractions as they came off an amino acid analyzer. The two peaks represent isodesmosine (left) and desmosine (right).

DISCUSSION

A major difficulty in the study of elastin from small animals has been the large sample of tissue required for gravimetric analysis (9, 10). This obstacle was overcome by the development of a technique capable of quantitating as little as 0.017 mg of elastin (0.5 nmol of desmosine and isodesmosine) (7). This method depends upon the insolubility of desmosine and isodesmosine in butanol:acetic acid:water, which allows an easy separation from other amino acids. Cysteine, lysine, and a few other very basic amino acids remain near the origin on paper chromatography, but these compounds separate from the desmosines on amino acid analysis.

By applying this methodology to analyze urine, we have been able to demonstrate that breakdown products of elastin are excreted in the urine for 3 days after the intratracheal instillation of pancreatic elastase. In all probability these elastin peptides were derived from the lungs, because elastin disappeared acutely from the lungs and because desmosines could not be detected in the urine of control animals. In separate studies, we have observed maximal excretion on the 1st or 2nd day after injection. Although we expected an early appearance, some animals produced little urine on the 1st day after injection. Very small amounts of desmosines were found in the urine 3 days after injection.

Only trace quantities of desmosines could be recovered from the feces of the injected hamsters. It is possible that these traces simply represent contamination of the feces by urine containing desmosine. Thus, excretion of elastin peptides via the fecal route seems minimal and probably inconsequential. Desmosines were not present in the feces of the control (uninjected) hamsters.

It is noteworthy that urinary recovery of elastin estimated to have been lost from the lungs averaged only 61%. Several factors may account for this incomplete recovery. Measurements of lung elastin loss and of urinary excretion were necessarily made on separate groups of hamsters. Conceivably, disparity in the intrapulmonary distribution and effects of intratracheally injected elastase between the groups may have caused this discrepancy. Secondly, it is possible that a portion of the elastin peptides are trapped in the body, rather than excreted. Body tissues, such as liver and spleen, were not examined for the presence of such peptides. Thirdly, there are probably some losses incurred during collection and processing. The recovery of standard desmosines added to urine before hydrolysis is also incomplete. This loss was the same whether the desmosines were added to urine from elastase-injected or control hamsters.

The possibility that desmosines were degraded in vivo seems unlikely because catabolism of intraperitoneally injected desmosine and isodesmosine could not be demonstrated. Urinary excretion was complete, and radiotracer studies revealed that these amino acids remained intact. In addition, no enzyme system capable of cleaving the elastin cross-links has ever been described.

It also seems unlikely that the desmosines were reincorporated into elastin or other peptides. Current knowledge of elastin synthesis holds that the cross-links form *de novo* as an integral part of the conversion of soluble tropoelastin to insoluble elastin (11, 12). Preformed cross-links thus cannot be incorporated into the elastin molecule. Nor are desmosines known to exist in any molecule other than elastin (13), thus eliminating this from consideration as an alternate mode of reutilization.

Analysis of urinary elastin peptides by gel filtration revealed a range in molecular weights of 9,000–27,000 daltons with predominance ≅13,000. This is consistent with the data of Senior et al. (14) who showed in vitro that peptides containing desmosine after digestion of elastin by pancreatic elastase were greater than 8,000 daltons, with 58% of the peptides ranging between 12,000 and 14,000 daltons. Peptides of this size should be cleared promptly by the kidneys (15).

The finding of elastin peptides in urine has potential implications which extend beyond the currently employed model of elastase-induced emphysema. To date, the study of elastin turnover has been limited by the lack of a marker which could be measured easily. Turnover studies usually have involved serial determinations of radiolabeled amino acids, such as lysine, proline, or glycine, that had been incorporated into the elastin of various tissues (16–18). Inherent limitations of these techniques include a requirement for actively growing tissues to incorporate the label, a determination that the label indeed remains in the elastin, and a need to sacrifice the animals under study. In addition, these methods lack sensitivity. Turnover of mature elastin is very slow, so that the duration of such studies must be quite prolonged if mature animals are used. Such techniques are obviously unsuitable for use in humans. The measurement of urinary desmosines offers a simple and noninvasive means for the detection of elastin turnover. It may be performed at any time during life, and requires only the collection of urine. It is applicable to humans, and, indeed, desmosines have previously been detected in normal human urine (19).

One might draw an analogy between urinary desmosines as an index of elastin catabolism and urinary hydroxyproline as an index of collagen catabolism. Hydroxyproline is essentially limited to collagen, and is present in measurable quantities in normal human urine (20). Urinary levels of hydroxyproline are elevated in acromegaly, hyperthyroidism, hyperparathyroidism, and protein wasting states. However, the parallel between hydroxyproline and desmosines is not precise. 30–50% of urinary hydroxyproline may be derived from soluble collagen (21), whereas desmosines come only from insoluble elastin. In addition, peptides containing hydroxyproline are absorbed from dietary sources (20). Such absorption of peptides containing desmosine seems unlikely because of their large molecular weight. Finally, unlike hydroxyproline, which may be degraded after release from collagen (21, 22), desmosines are very stable and do not appear to be catabolized in the body. Therefore, desmosines would seem to be suitable for quantitative measurements of elastin turnover. It has been suggested (23, 24), however, that a considerable proportion of elastin turnover may involve the degradation of immature elastin in which desmosines are either absent or present in reduced numbers. Such turnover would not be reflected by urinary desmosine measurements.

In summary, we have shown that elastin turnover can be measured by quantitating the desmosine content of the urine. The elastin turned over in the hamster model used in these studies resulted from an acute injury to the lung. Although there is a potential limitation of the method in situations of very slow elastin turnover we feel this approach can be extended to studies of growth and development and in diseases of connective (elastic) tissue.

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