Distribution of Elastin in Hamsters and the Turnover Rates of Different Elastin Pools (44118)

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Abstract. **Desmosine (DES) and isodesmosine (IDES) concentration in the urine can be used as a noninvasive method of assessing degradation of mature elastin in normal and pathologic states. The present study was undertaken to determine the distribution of elastin among organs and tissues of normal hamsters, and to determine the turnover rates of two elastin-containing organs (lung, thoracic aorta) as a reflection of their contributions to DES and IDES excretion in the urine. Hamsters were metabolically labeled at 5 days of age with ^{14}C-lysine and studied at 1.5, 4.5, 8, and 12 months of age. The aorta DES+IDES–associated radioactivity did not change significantly over the age span of 1.5–12 months. Lung DES+IDES–associated radioactivity decreased with a half-life of 420 days. Measurement of DES+IDES pools in other tissues, with relatively low concentrations of elastin, was carried out by the isotope dilution technique. At 12 months of age, the head and paws pool, skin, skeletal muscle, gastrointestinal tract, heart-liver-kidney-spleen pool, lungs, and thoracic aorta represented 37%, 28%, 13%, 11%, 6%, 4%, and 1%, respectively, of total body DES+IDES. The organs with the highest DES+IDES–specific radioactivity at 12 months were heart-liver-kidney-spleen, lung, and gastrointestinal tract, with 310, 217, and 217 dpm/nmol, respectively. Skin had the lowest specific radioactivity, with 90 dpm/nmol. The specific radioactivity of DES+IDES in urine was 62 dpm/nmol at 12 months, down from 251 dpm/nmol at 1.5 months. These data clearly indiciate that non-lung tissues contain a high proportion of the total body DES+IDES and suggest that pathology in these other pools of DES+IDES could result in significant elevation of urinary DES+IDES. Nevertheless, the relatively high specific radioactivity of DES+IDES in lung elastin as compared with urine makes monitoring labeled urinary DES+IDES in this animal model a sensitive tool for assessing elastin degradation in experimental lung disease.**

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Development of the elastase-antielastase hypothesis of emphysema was a milestone in the search to understand the pathogenesis of emphysema (1). The concept of an imbalance of elastases and antielastases has been extended to several other pulmonary diseases, such as cystic fibrosis and adult respiratory distress syndrome (2, 3). The finding in urine of elevated concentrations of elastin metabolites, such as desmosine (DES) and isodesmosine (IDES), was evidence for proteolytic destruction of elastin. Elevated levels of DES and IDES have been found in the urine of patients with cystic fibrosis (4), adult respiratory distress syndrome (5), and chronic obstructive pulmonary disease (6).

Estimates of lung elastin turnover rates in hamsters and healthy neversmoking humans suggested that lung elastin was a minor source of urinary DES in normal individuals (7). Other tissue sources that may contribute to urinary DES and IDES have not been established. For measurement of urinary DES to be used in the clinical assessment of disease, other sources of urinary DES and IDES should be identified, and their rate of turnover measured. High rates of turnover of these

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other pools could also produce elevated levels of urinary DES. The present study was designed to obtain information on the DES content of various organs and tissues in normal hamsters, and their rates of turnover.

Materials and Methods

Experimental Design. Turnover of elastin in normal hamsters was studied by measuring levels of radioactivity in elastin protein as well as DES+IDES and lysyl residues of elastin formed during metabolic labeling of neonatal animals. These levels of radioactivity were measured as the animals aged, in conjunction with the appearance of DES+IDES in the urine. The DES+IDES in the urine was derived from more than one tissue. The importance of each source can be identified by quantifying the amount and turnover rate in each tissue. Two equations can be written:

nmol Urine DES+IDES/day
= Excretion of Lung DES+IDES in nmol/day
+ Excretion of Aorta DES+IDES in nmol/day
+ Excretion of All-Other DES+IDES in nmol/day, (1)

and

dpm Urine DES+IDES/day
= Excretion of Lung DES+IDES in nmol/day
× dpm/nmol DES+IDES
+ Excretion of Aorta DES+IDES in nmol/day
× dpm/nmol DES+IDES
+ Excretion of All-Other DES+IDES in nmol/day
× dpm/nmol DES+IDES, (2)

where "All-Other" refers to tissue pools of DES+IDES other than lung and aorta.

Excretion of DES+IDES derived from lung and aorta were calculated from the respective DES+IDES pool sizes in nanomoles times the first-order rate constants, k, in day^{-1}, for the decreases in radioactivity of the respective DES+IDES and elastin-specific lysyl residue pools. Using data from the four time points, the first-order rate constants were determined using least squares analysis and the equation $kt = \ln(C_0/C)$, where C_0 was the amount at the initial time, C was the amount at a later time, and t was the time interval between the initial and later time. The half-life ($t_{1/2}$) was calculated as $0.693/k$.

Experimental Protocol. Five-day-old hamster pups were intraperitoneally injected with 0.1 mCi of ^{14}C-lysine. Approximately equal numbers of male and female hamsters were then studied at 1.5 (n = 16), 4.5 (n = 16), and 8 months (n = 8). At 12 months (n = 10), 8 female and 2 male hamsters were studied. Hamsters were maintained on standard 5001 Purina Rodent Laboratory Chow. One week before urine collection and throughout the period of urine collection, hamsters were maintained on a DES- and IDES-free Purina test diet (7). At each study period, urine was collected every other day for 3 days. Hamsters were fed on alternate days outside of the metabolic cages to minimize the amount of food powder dropping into the urine collection chamber.

After urine collection, hamsters were euthanized with pentobarbital followed by removal of lungs (minus extrapulmonary vessels and airways) and aorta down to the diaphram. Hair from the skin was removed, but not analyzed. Skin from the torso, neck, and legs were removed. Skin from the paws and face were not included in the analysis of skin tissue because of the difficulty in removing it. Careful removal of the small amount of adherent muscle tissue from four skins of 4.5-month-old hamsters and pooling and analysis for DES+IDES provided an estimate of the amount of DES+IDES in the skin pool that derived from adherent muscle tissue. Additional tissue pools were analyzed from three 12-month-old female hamsters: head (including the skull and skin) and paws; skeletal muscle; stomach and intestines (GI tract); and heart, liver, kidneys, and spleen (the visceral pool). Neither testes from male hamsters nor skeletons from any of the animals were analyzed.

Measurement of Elastin and DES+IDES in Tissues. *Measurement of elastin and DES+IDES in aorta and lung by hot alkali method.* The lung and aorta, relatively high in elastin concentration, were each homogenized at 2°C, and aliquots were removed for isolation of elastin by the hot alkali method (8). The amount and amino acid composition of elastin were determined by amino acid analysis (Model 6300 Amino Acid Analyzer; Beckman, Palo Alto, CA). The eluant from the amino acid analyzer was collected in 1-min fractions and assessed for radioactivity. Specific radioactivity for DES+IDES and lysine were calculated from this data.

Measurement of DES+IDES in all other tissues. The hot alkali method for purification of elastin is unsuitable for tissues in which elastin is only a minor component. The DES content of the remaining tissue pools, which had a relatively low concentration of elastin, was analyzed by the isotope dilution technique as previously described, but using ^{3}H-DES to spike the samples and double isotope counting (Model 1900 TR; Packard, Meriden, CT) (9).

DES+IDES–associated radioactivity in each tissue pool, measured by the isotope dilution technique, was calculated by multiplying the nmol of DES+IDES in that pool by the specific radioactivity of DES+IDES.

Isotope Dilution Assay for Urinary DES and IDES. The isotope dilution technique we have devised for assessment of urinary DES (7) allows adequate cleanup of the acid hydrolyzed sample with internal correction for the variable losses that occur among samples. Briefly, urine samples were clarified by centrifuga-

tion (30,000 *g*). Aliquots were taken for the creatinine concentration determination, and the remainder was spiked with known amounts of ^{3}H-DES. The urine samples were combined with an equal volume of 12 *N* HCl and refluxed under N_2 at 110°C to hydrolyze peptide bonds. The residue was dried and subjected to extensive gel filtration in order to remove interfering contaminants and to obtain fractions highly enriched in the relatively high molecular weight components.

DES and IDES were separated by approximately 0.7 min, using a modification of the HPLC method of Black and co-workers (10) that employs reverse-phase ion-pairing on a C_{18} column (7). Double label counting of urine fractions (^{3}H/^{14}C) allowed us to compute recovery of DES and IDES in urine samples that had been spiked with ^{3}H-DES.

Values for DES were calculated using an isotope dilution calculation and normalized for the creatinine content of the sample (7). Values for IDES were calculated using the same dilution factor as found for DES (7). DES and IDES values were expressed as micrograms per gram of creatinine.

Statistical Analysis. Data are presented as the mean ± SD. Using Statview 4.01 (Abacus Concepts, Berkeley, CA), comparisons between two groups were made with an unpaired *t* test. Comparisons involving three groups or more were made using analysis of variance and the Scheffe test. Probability values less than 0.05 were considered significant.

Results

Elastin and DES+IDES Content of Lung and Aorta. Between 1.5 and 12 months of age, lung (Fig. 1A) and aorta (Fig. 2A) elastin increased by 44% and 60%, respectively, and lung and aorta DES+IDES increased by 74% and 166%, respectively; differences were statistically significant ($P < 0.05$). Mean body weight increased by 83% during the same interval, from 77 to 141 g ($P < 0.05$).

Comparison of DES+IDES Content of Tissue Pools. The elastin content of skin; head and paws; skeletal muscle; GI tract; and viscera could not be determined directly since elastin could not be readily isolated from these tissues. The DES+IDES contents of

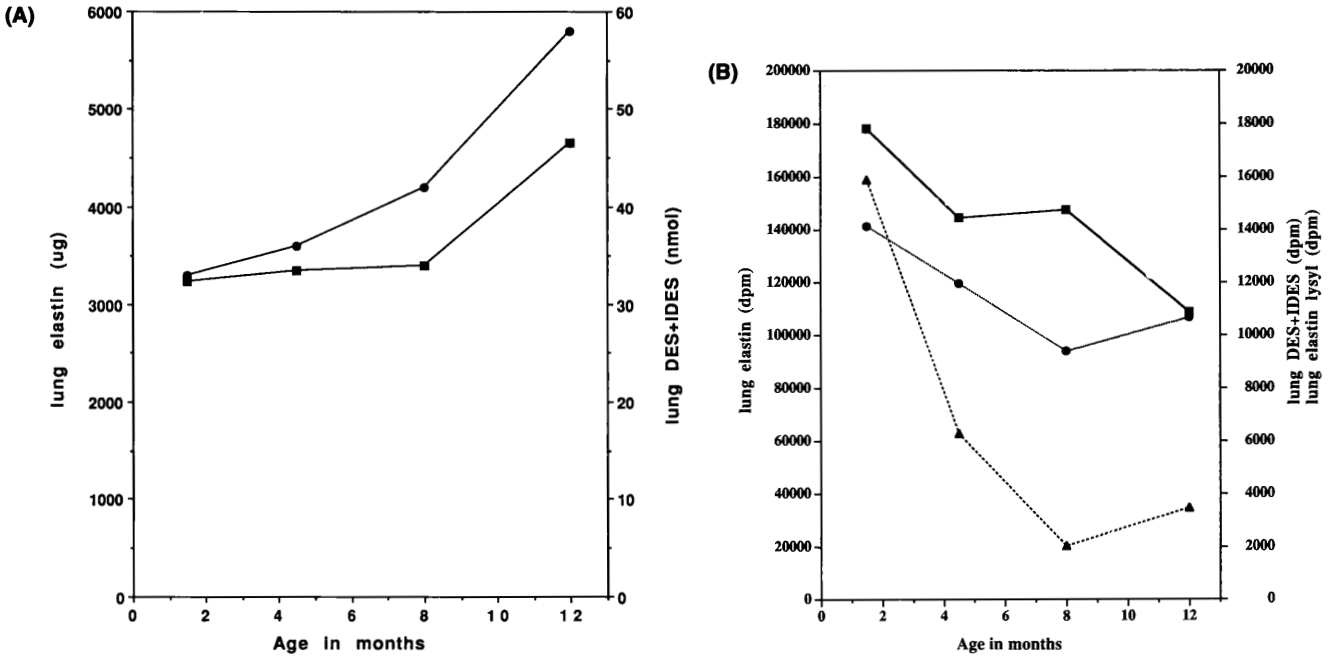

Figure 1. (A) Effect of age on the amount of elastin (■) and DES+IDES (●) in hamster lung. Values for lung elastin were 3231 ± 611 ($n = 16$), 3350 ± 242 ($n = 16$), 3396 ± 877 ($n = 8$), and 4653 ± 2235 ($n = 9$) μg for 1.5-, 4.5-, 8-, and 12-month-old hamsters, respectively. The 12-month values were different from the 1.5- and 4.5-month but not the 8-month values ($P < 0.05$, Scheffe test). Mean values for lung DES+IDES were 33.2 ± 7.8 ($n = 16$), 35.7 ± 8.6 ($n = 16$), 42.3 ± 6.9 ($n = 8$), and 57.7 ± 21.9 nmol for 1.5-, 4.5-, 8-, and 12-month-old hamsters, respectively. The mean value for the 1.5- and 4.5-month-old hamsters were different from that for the 12-month-old hamsters ($P < 0.05$, Scheffe test). (B) Effect of age on the lung elastin-associated (■), DES+IDES–associated (●), and elastin lysyl residue-associated (▲) radioactivity in hamsters metabolically labeled at 5 days of age. Values for lung elastin-associated radioactivity were 178,167 ± 39,574 ($n = 16$), 144,632 ± 26,447 ($n = 16$), 147,685 ± 30,656 ($n = 8$), and 108,754 ± 26,451 ($n = 9$) dpm for 1.5-, 4.5-, 8-, and 12-month-old hamsters, respectively. The mean values for the 1.5-month-old hamsters were significantly different from the 4.5- and 12-month-old hamsters ($P < 0.05$, Scheffe test). Values for lung elastin DES+IDES–associated radioactivity were 14,135 ± 4,086 ($n = 16$), 11,956 ± 2,444 ($n = 16$), 9,397 ± 1,672 ($n = 8$), and 10,664 ± 3,569 ($n = 7$) dpm for 1.5-, 4.5-, 8-, and 12-month-old hamsters, respectively; the mean values for the 1.5- and 8-month-old hamsters were significantly different from each other ($P < 0.05$, Scheffe test). Values for lysyl residues of elastin in lung were 15,894 ± 5,548 ($n = 16$), 6,282 ± 1,122 ($n = 16$), 2,040 ± 460 ($n = 8$), and 3,486 ± 2,036 ($n = 7$) dpm for 1.5-, 4.5-, 8-, and 12-month-old hamsters, respectively. The mean value for the 1.5-month-old hamsters was different from those for 4.5-, 8-, and 12-month-old hamsters ($P < 0.05$, Scheffe test).

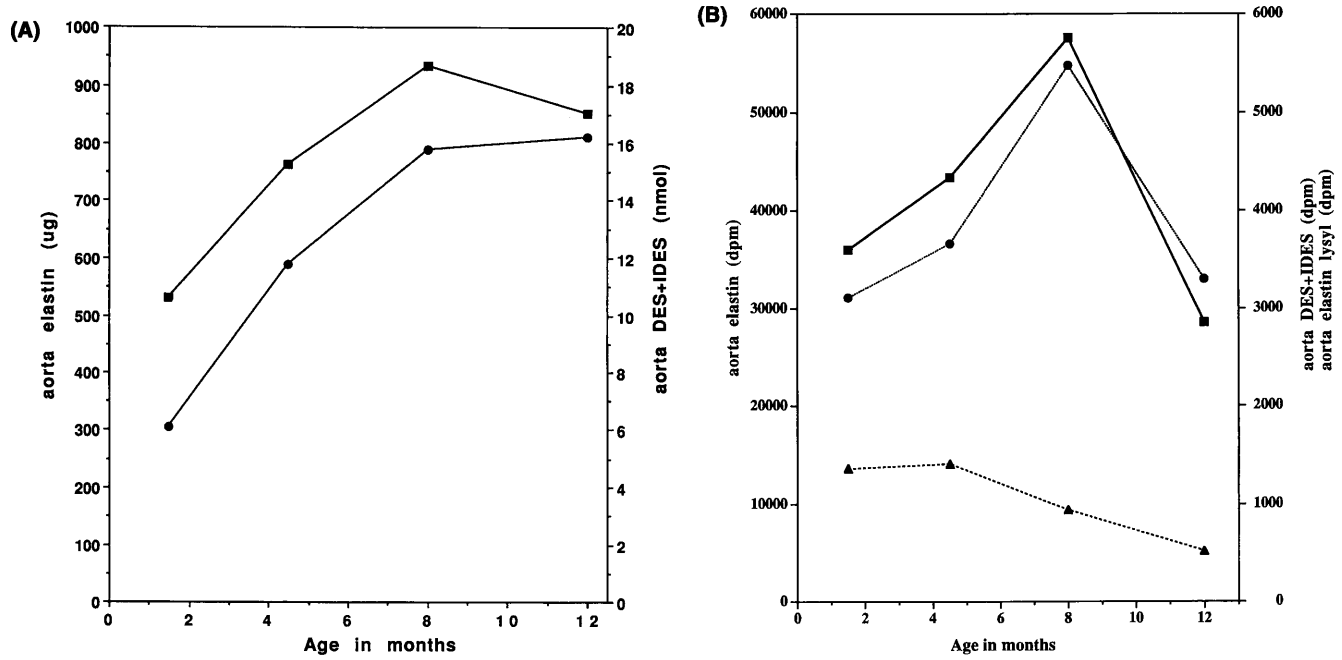

Figure 2. (A) Effect of age on the amount of elastin (■) and DES+IDES (●) in hamster aorta. Aorta elastin values were 532 ± 159 (n = 16), 764 ± 200 (n = 16), 933 ± 283 (n = 8), and 851 ± 191 (n = 6) μg, respectively. The mean value for the 1.5-month-old hamsters was different from that for the 4.5-, 8-, and 12-month-old hamsters ($P < 0.05$, Scheffe test). Mean values for aorta elastin DES+IDES were 6.1 ± 1.7 (n = 16), 11.8 ± 3.9 (n = 16), 15.8 ± 3.9 (n = 8), and 16.2 ± 5.4 (n = 9) nmol, respectively. The mean value of hamster aorta elastin DES+IDES for the 1.5-month-nold hamsters was different from those for the 4.5-, 8-, and 12-month-old hamsters ($P < 0.05$, Scheffe test). (B) Effect of age on the aorta elastin-associated (■), DES+IDES–associated (●), and elastin lysyl residue-associated (▲) radioactivity in hamsters metabolically labeled at 5 days of age. Values for aorta elastin-associated radioactivity were 35,996 ± 13,982 (n = 16), 43,396 ± 12,559 (n = 16), 57,558 ± 16,829 (n = 8), and 28,596 ± 7,386 (n = 9) dpm for 1.5-, 4.5-, 8-, and 12-month-old hamsters, respectively. The mean value for the 1.5-month-old hamsters was different from that for the 8-month-old hamsters and the mean value for the 8-month-old hamsters was different from that for the 12-month-old hamsters ($P < 0.05$, Scheffe test). Values for aorta elastin DES+IDES–associated radioactivity were 3,110 ± 1,246 (n = 16), 3,660 ± 1,137 (n = 16), 5,475 ± 2,040 (n = 8), and 3,301 ± 935 (n = 9) dpm for 1.5-, 4.5-, 8-, and 12-month-old hamsters, respectively. The mean value for the 1.5-month-old hamsters was different from that of the 8-month-old hamsters, that for the 4.5-month-old hamsters was different from that for the 8-month-old hamsters, and that for the 8-month-old hamsters was different from the 12-month-old hamsters. Values for lysyl residues of elastin in aorta were 1,360 ± 712 (n = 16), 1,407 ± 445 (n = 16), 940 ± 480 (n = 8), and 520 ± 232 (n = 9) dpm for 1.5-, 4.5-, 8-, and 12-month-old hamsters, respectively. The mean value for the 1.5- and 4.5-month-old hamsters were different from that for the 12-month-old hamsters ($P < 0.05$, Scheffe test).

these tissue pools are compared with each other and the DES+IDES content of aorta and lung at 12 months (Table I). Aorta and lung represented the smallest tissue pools of DES+IDES, 1% and 4% of the total, respectively; head and paws, and skin were highest at 37% and 28%, respectively; and skeletal muscle, GI tract, and viscera were intermediate, ranging from 13% to 6% of the total. The amount of skin-associated DES+IDES more than doubled between 1.5 and 4.5 months of age and then remained constant (Fig. 3).

Table I. Ranking of DES+IDES Distribution in 12-Month-Old Hamsters

Tissues	DES+IDES (nmol)	DES+IDES (dpm/nmol)	DES+IDES (dpm)
Head and paws	424 ± 87 (37%)[a]	138 ± 10	58,159 ± 7,671 (34%)[a]
Skin	320 ± 27 (28%)	90 ± 15	28,530 ± 3,102 (17%)
Skeletal muscle	155 ± 25 (13%)	158 ± 27	24,523 ± 6,408 (14%)
GI tract	124 ± 32 (11%)	217 ± 26	26,490 ± 4,697 (16%)
Viscera	64 ± 18 (6%)	310 ± 47	19,656 ± 4,972 (12%)
Lungs	50 ± 10 (4%)	217 ± 24	11,100 ± 3,303 (6%)
Aorta	12 ± 4 (1%)	197 ± 26	2,317 ± 529 (1%)
Total	1,149 ± 153 (100%)		170,775 ± 7,561

Note. Values are the mean ± SD for three hamsters whose organs were analyzed. Uterus, which contains 1.4 ± 0.6 (n = 8) nmol DES+IDES in 4.5-month-old hamsters, was not analyzed in 12-month-old hamsters.
[a] Percentage of total.

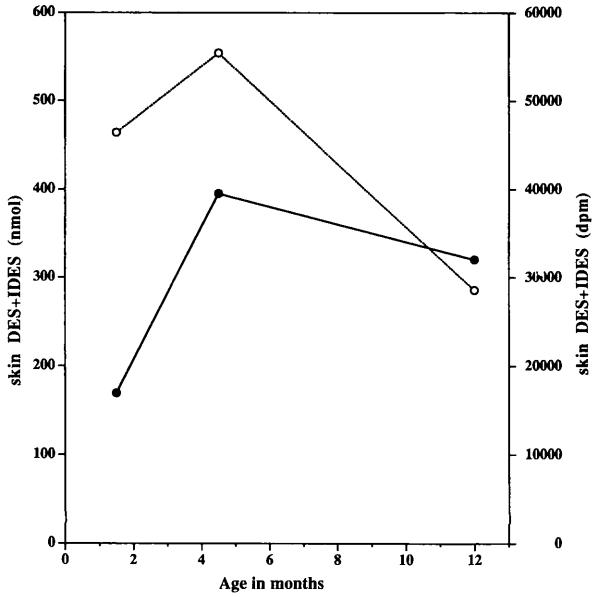

Figure 3. Effect of age on the amount of DES+IDES (●) and the DES+IDES–associated radioactivity (○) in the skin of hamsters metabolically labeled at 5 days of age. Mean values for skin DES+IDES at 1.5, 4.5, and 12 months were 169 ± 92 (n = 2), 395 ± 79 (n = 3) and 320 ± 27 (n = 3) nmol per animal. Values for skin DES+IDES radioactivity were 46,410 ± 30,335 (n = 2), 55,417 ± 17,355 (n = 3), and 28,530 ± 3,101 (n = 3) dpm per animal for 1.5-, 4.5-, and 12-month-old hamsters, respectively.

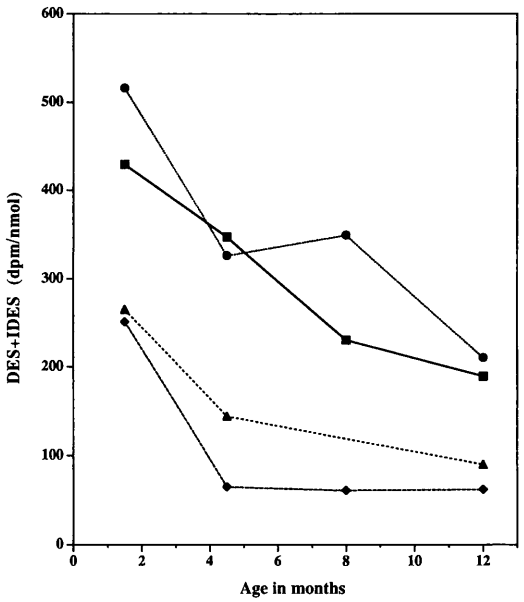

Figure 4. Effect of age on the specific radioactivity of DES+IDES in lung (■), aorta (●), skin (▲), and urine (◆). Mean values of lung DES+IDES specific radioactivity were 429 ± 96 (n = 16), 347 ± 95 (n = 16), 230 ± 61 (n = 8), and 189 ± 37 (n = 7) dpm/nmol for 1.5-, 4.5-, 8-, and 12-month-old hamsters, respectively. The mean value for 1.5-month-old hamsters was different from that for the 8-month-old hamster ($P < 0.05$, Scheffe test). Mean values of aorta DES+IDES–specific radioactivity were 516 ± 169 (n = 16), 326 ± 94 (n = 16), 349 ± 110 (n = 8), and 210 ± 40 (n = 9) dpm/nmol for 1.5-, 4.5-, 8-, and 12-month-old hamsters, respectively. The mean value for 1.5-month-old hamsters was different from those for the 4.5-, 8-, and 12-month-old hamsters ($P < 0.05$, Scheffe test). Mean values of skin DES+IDES–specific radioactivity were 265 ± 35 (n = 2), 144 ± 50 (n = 3), and 90 ± 15 (n = 3) dpm/nmol for 1.5-, 4.5-, and 12-month-old hamsters, respectively. Mean values of urine DES+IDES–specific radioactivity for hamsters were 251 ± 56 (n = 16), 65 ± 23 (n = 16), 61 ± 22 (n = 6), and 62 ± 21 (n = 7) dpm/nmol for 1.5-, 4.5-, 8-, and 12-month-old hamsters, respectively. The mean values for 1.5-month-old hamsters was different from those for the 4.5-, 8-, and 12-month-old hamsters ($P < 0.05$, Scheffe test).

Decrease or Radiolabeled Elastin in Lung and Aorta with Age. Lung elastin-associated radioactivity and DES+IDES–associated radioactivity decreased, due to metabolic turnover, with a half-life of 447 and 420 days, respectively (Table II and Figure 1B). Aorta elastin-associated radioactivity and aorta DES+IDES–associated radioactivity did not decrease significantly (Fig. 2B). A relatively rapid decrease of radioactivity specific to lung elastin lysyl residues was found with a half-life of 87 days or 5 times faster than total lung radioactivity or lung DES+IDES radioactivity (Table II and Fig. 1B). In aorta, lysyl-associated radioactivity in elastin decreased with a longer half-life of 192 days (Table II and Fig. 2B). In neither case did the lysyl content of elastin change with age (10 ± 2, n = 16, and 14 ± 5 residues/1000, n = 4, for lungs of 1.5- and 12-month-old hamsters, respectively, and 7 ± 3, n = 16, and 7 ± 6, n = 9, for aortas of 1.5- and 12-month-old hamsters, respectively).

Specific Radioactivity of DES+IDES in Lung, Aorta, Skin, and Urine. At 1.5 months of age the spe-

Table II. First-Order Rate and $t_{1/2}$ of Turnover of Elastin in Hamster

Tissue	Marker	r	k (day^{-1})	$t_{1/2}$ (day)
Lung	Elastin protein radioactivity	0.93	0.00155	447
Lung	Elastin DES+IDES radioactivity	0.78	0.00165	420
Lung	Elastin lysyl residue radioactivity	0.80	0.0079	87
Aorta	Elastin protein radioactivity	0.23	Indeterminate	
Aorta	Elastin DES+IDES radioactivity	0.06	Indeterminate	
Aorta	Elastin lysyl residue radioactivity	0.94	0.00362	192

Note. First-order rates were calculated as described in Materials and Methods. The correlation coefficient (r) is listed for each least squares plot from which the slope (k) was determined. The half-life ($t_{1/2}$) was calculated as 0.693/k.

cific radioactivity of lung and aorta DES+IDES was 429 and 516 dpm/nmol, respectively (Fig. 4). By 12 months of age, with the excretion of radiolabeled DES+IDES as well as dilution with new nonradiolabeled DES+IDES, the specific radioactivities decreased by over 50% (189 and 210 dpm/nmol, respectively). The specific radioactivity of urinary DES+IDES in hamsters decreased from 251 dpm/nmol in 1.5-month-old hamsters to 65 dpm/nmol in 4.5-month-old hamsters and remained between 61 and 62 dpm/nmol in 8 and 12-month-old hamsters (Fig. 4). The specific radioactivity of skin decreased from 265 ± 35 dpm/nmol at 1.5 months to 144 ± 50 at 4.5 months and 90 ± 15 at 12 months, similar to the values for DES+IDES in urine.

Mean values of urinary DES+IDES excretion were 0.501 ± 0.163 (16), 0.348 ± 0.078 (16), 0.485 ± 0.173, and 0.269 ± 0.115 nmol/day for 1.5-, 4.5-, 8-, and 12-month-old hamsters, respectively. The mean value for 1.5-month-old (young adult) hamsters was significantly greater than those for the 4.5- and 12-month-old hamsters and the mean value for 8-month-old hamsters was greater than that for 12-month-old hamsters ($P < 0.05$, Scheffe test). Normalized for creatinine excretion, 1.5-month-old hamsters excreted 416 nmol DES+IDES/g creatinine, and mature adult hamsters excreted 124–211 nmol/g creatinine.

Discussion

The present study was undertaken to obtain information on tissue sources of elastin and the turnover rates of these pools, which contribute to urinary DES and IDES. Our findings on the slow rate of decrease of radioactivity associated with elastin and with DES+IDES residues of elastin in lung and aorta of metabolically labeled hamsters are consistent with other studies that used small experimental animals. Using metabolic labeling with ^{14}C-lysine of young rats, mice, and quail, Rucker and co-workers found that the best estimate of elastin turnover in aorta and lungs was only quantifiable in years (11–14). The present study extends those studies by looking at excreted DES+IDES at four time points, skin at three time points, and other tissues at one time point.

Using the kinetic constants determined for disappearance of a tissue pool of DES+IDES-associated radioactivity, one can calculate the expected contribution of a DES+IDES tissue pool to urinary DES+IDES. Assumptions include: (i) The decrease in radioactivity of a tissue pool of elastin is first order. (ii) Radiolabeled DES and IDES are catabolized at the same rate as unlabeled DES and IDES. (iii) DES and IDES turnover at the same rate. It is known that DES is not metabolized to any measurable extent, but rather is excreted in the urine (15). For 1.5-month-old hamsters the amount of urinary DES+IDES per day derived from lung, based on the decrease in lung DES+IDES radioactivity, was 0.055 nmol ($-kC = -0.00165$ day^{-1} × 33.2 nmol lung DES+IDES), representing 11% of the 0.501 nmol DES+IDES total in urine. The values for 4.5-, 8-, and 12-month-old hamsters were 0.059, 0.070, and 0.095 nmol, respectively, representing 17%, 14%, and 35% of the urinary DES+IDES derived from lung, respectively. Thus, on the average, 19% of urinary DES+IDES is lung derived. Yet lung DES+IDES represented only 4% of the total present in the animal at 12 months and this estimate excludes any DES+IDES present in tissues that were not analyzed, such as the skeleton and testes.

Aorta elastin appears to turnover more slowly than lung (Figs. 1 and 2). We could not detect a decrease in aorta elastin-associated radioactivity or aorta DES+IDES–associated radioactivity. The mean rate of turnover of all DES+IDES (1149 nmol) in the 12-month-old hamsters, based on the rate of excretion of DES+IDES, was 1149 nmol/0.3 nmol excreted per day or a mean half-life of 5.3 years for DES+IDES.

The variability of the skin DES+IDES data may be due, in part, to the presence of variable amounts of skeletal muscle tissue adherent to the skin. At the beginning of this study we did not appreciate the DES+IDES content of skeletal muscle. Adherent muscle tissue from four skins of 4.5-month-old hamsters contained 22 nmol of DES+IDES per hamster compared with 395 nmol of DES+IDES in the cleaned skin.

There was a more rapid decrease in lysyl residue-associated radioactivity (5-fold in the case of lung), compared with DES+IDES–associated radioactivity or total elastin-associated radioactivity. Since the lysyl residue content of lung and aorta elastin did not decrease with age, possible explanations include the continued utilization of older lysyl residues for crosslinks as new tropoelastin molecules, containing few radiolabeled lysyl residues, are added to the elastic fiber. It has been suggested that the continued growth of elastic fibers in the extracellular matrix is the result of the deposition of newly synthesized material on preexisting elastic fibers (16). Another possibility is that turnover of elastin can occur with the preferential loss of the lysines, while the lysine-derived cross-links are more resistant to removal (12). Unless slowly exchanging elastin-specific pools of lysyl residues exist, reutilization of ^{14}C-lysine in older animals may have been unimportant. Lysine-specific radioactivity in the urine of 1.5-month-old hamsters was only 4 ± 2 dpm/nmol ($n = 3$) and was not measurable in urine and plasma lysyl residues from 4.5-month-old hamsters.

The extent of labeling of different tissues in the neonates should depend on the rate of elastin synthesis at that time. DES+IDES in lung elastin exhibited a high level of specific radioactivity due to the high level of metabolic labeling of the lungs during the neonatal period. High levels of elastin promoter expression in

the lungs of 5-day-old trangenic mice has been demonstrated (17).

The finding in 12-month-old hamsters of 424 nmol DES+IDES in the head and paws or 37% of total DES+IDES was unexpected. Separate studies indicated that more than 93% of the DES+IDES in that pool was found in the head and less than 1 nmol DES+IDES was present in excised brain tissue (not shown). Possible specific tissues contributing to this amount of DES+IDES in the head include the neck ligaments, bone and cartilage, cheek pouches, and skin that was not removed.

There is little literature on the DES content of tissues other than lung, aorta, skin, and uterus. In a study of beef muscle, we found approximately 25 nmol DES+IDES/g wet wt, or an elastin concentration of around 0.1% of wet weight (9). A portion of the muscle DES+IDES pool may be vascular in origin.

A portion of the decrease in lung, aorta, and skin DES+IDES–specific radioactivity with age is associated with and can be explained by increases in the amount of DES+IDES present; between 1.5 and 12 months of age, the increase was 74% for lung, 166% for aorta, and 89% for skin. At 12 months of age the specific radioactivity of DES+IDES in skin was 90 dpm/nmol, the lowest DES+IDES specific radioactivity of the tissue pools we examined, compared with 60 dpm/nmol for urine (Table I). Skin may have contributed a large portion of the urinary DES+IDES. The organs with the highest specific radioactivity at 12 months were viscera, lung, and gastrointestinal tract with 310 ± 47, 217 ± 24, and 217 ± 26 dpm/nmol, respectively.

Although we have expressed urinary excretion data both as nanomoles per gram of creatinine and nanomoles per day, recovery of urine in metabolic cages is prone to error. Hamsters excrete only 2–3 ml of urine per day. Measurement of the specific radioactivity of crosslink amino acids in urine is not subject to these errors in urine recovery. The specific radioactivity of the urine DES+IDES was highest in 1.5-month-old hamsters (251 dpm/nmol) and leveled off in 4.5-month and older hamsters at 60–65 dpm/nmol. Since the mean value for DES+IDES dpm/nmol in lung and aorta elastin did not drop below 189 and 349 dpm/nmol, respectively, there likely existed source(s) of DES/IDES in 4.5-month and older hamsters with a specific radioactivity of 61 dpm/nmol or less that contributed significantly to the urine pool. We did not identify any sources of DES+IDES–specific radioactivity lower than 90 dpm/nmol.

As mentioned earlier, these calculations are based on the assumption that the contribution of radiolabeled DES and IDES to urine is the same as nonlabeled DES and IDES. In a complex organ such as the lung, this assumption is probably not totally correct. Perhaps the elastin in vessel walls, large airways, and alveolar walls, for example, turns over at different rates. Therefore, the specific activity of the degraded DES and IDES would be more representative of that specific tissue pool. Nevertheless, urinary DES+IDES–specific radioactivity will likely increase following lung elastin injury in the hamster.

In summary, the question of where the baseline DES+IDES in urine originates has not been answered, but the extent of the total tissue DES+IDES pool and the approximate location of the previously unreported pools have been identified.

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