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Effect of Papain-Induced Emphysema on Canine Pulmonary Elastin (40898)[1]

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Abstract. Experimental emphysema was induced in eight beagles by repeated administration of crude papain via aerosol and intratracheal instillation. Significant increases in mean linear intercept in the lungs of the papain treated dogs were consistent with morphological evidence of ruptured alveolar septa. The proportions of elastin in the parenchymal connective tissue were calculated from the desmosine and isodesmosine content of the total connective tissue and of purified elastin from the same specimens. Mean pulmonary elastin proportions determined 2 weeks after the last dose of papain in four dogs with experimental emphysema were only 4.52% ± 1.84 whereas mean pulmonary elastin proportions in four control dogs were 14.42% ± 3.55. In two dogs sacrificed 3 months after the termination of the insult, mean pulmonary elastin proportions were not significantly different from those in the control dogs. Since the elastolytic activity of papain is very low these data suggest that the elastin may be degraded *in vivo* by other mechanisms involving enhancement of endogenous elastase activity.

The first induction of experimental emphysema was accomplished by Gross *et al.* (1) by intratracheal injection of papain into rats. Subsequently similar methods were used to produce emphysema in hamsters (2–5), rats (6–8), and dogs (9–11) by the administration of papain (2, 3, 6, 7, 9, 10), pancreatic elastase (4, 5), polymorphonuclear leukocyte and alveolar macrophage cell homogenates (11), and neutral proteases of microbial origin (8). Morphological observations of the lesions indicated that damage was confined to the elastic fibers and that only enzymes with elastolytic activity were capable of inducing emphysema (8, 12). Other proteolytic enzymes such as trypsin or collagenase (4, 12) did not produce emphysema and crystalline papain devoid of elastolytic activity had no effect (13). Specific assays for changes in elastin content were only performed in a few cases. Kuhn *et al.* (14) found a diminution in lung elastin content to one-quarter the initial concentration 24 hr after a single endotracheal injection of pancreatic elastase into hamsters. However, after 2 months, regeneration of elastin had restored the amount to its original level although the architecture of the newly laid down elastin was altered. The detection of desmosine containing peptides in the urine of such hamsters (15) lent further support to the concept of transient destruction of elastin. Kilburn *et al.* (3) did not observe alterations in total lung protein, collagen, or elastin following emphysema induction by an endotracheal dose of 1 mg purified papain in hamsters. In the present study significantly higher doses of papain were administered in multiple doses to dogs. Crude papain has a measurable but low elastolytic activity and it seemed of interest to determine its effect on the elastin content of lung connective tissue. Elastin proportions were calculated from the number of residues of the elastin-specific cross-linking amino acids desmosine and isodesmosine in the crude connective tissue moiety (15–17).

Materials and methods. Ten beagles weighing between 11 and 13 kg, previously conditioned by the vendor to assure absence of internal parasites, were subjected to blood, chemical, and x-ray tests including a special test for the absence of heart worm, to indicate a healthy state. Four of the dogs were kept as controls, the other six were anesthetized by intravenous administration of sodium pentobarbital 20–30

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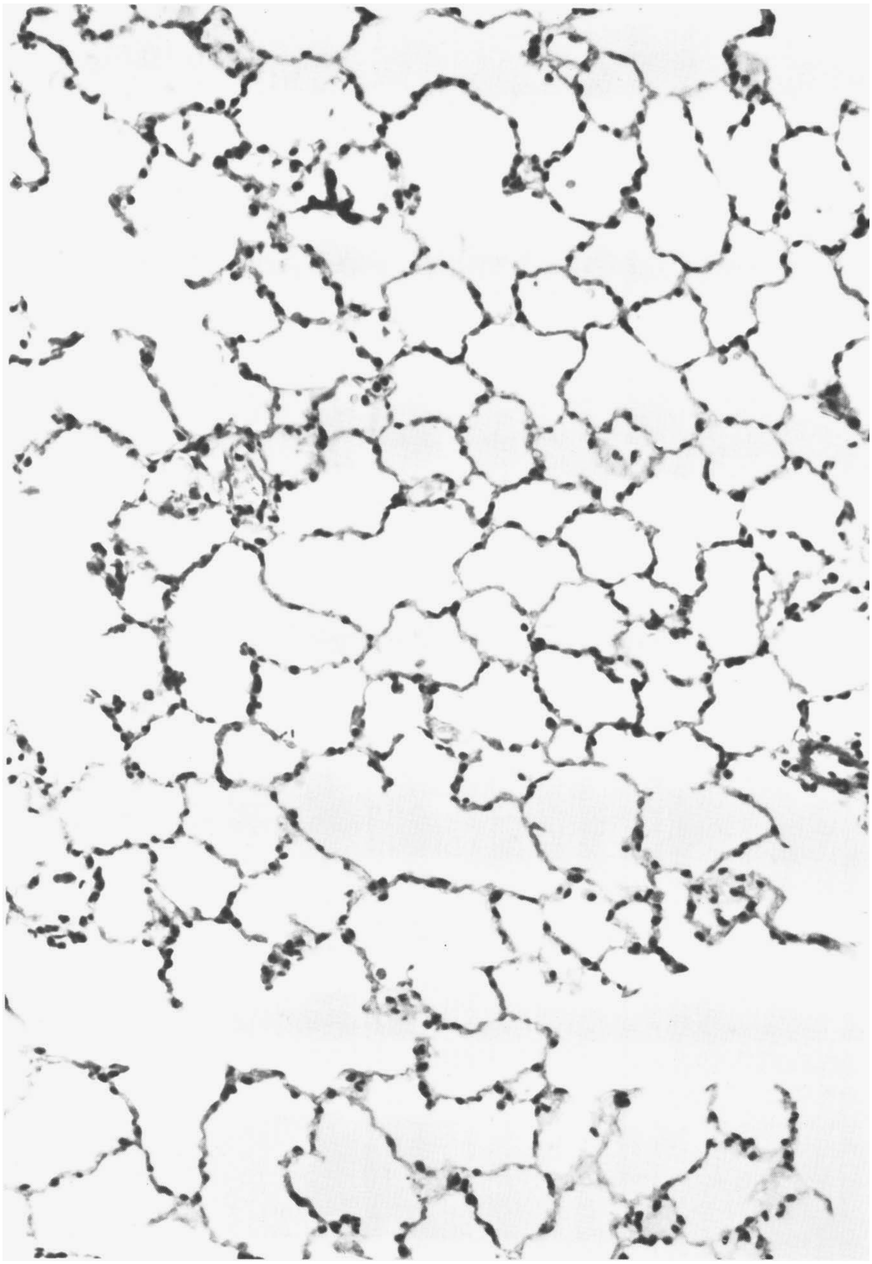

FIG. 1. Paraffin section from the left lung of a control dog. Hematoxylin and eosin. ×235.

mg/kg. Cuffed intratracheal tubes were inserted and emphysema induced by a combination of the methods described by Pushpakom *et al.* (9) and by Marco *et al.* (10) modified as follows to increase exposure to the enzyme: 2.5 ml of a 16% solution of papain (Sterling–Winthrop Laboratories, 300,000 units/mg)[2] in 0.9% sodium chloride was nebulized and the aero-

[2] 0.087 elastolytic units/mg determined with elastin–orcein or fibrous elastin substrates calibrated against pancreatic elastase (Elastin Products, St. Louis, Mo.) of 60 units/mg.

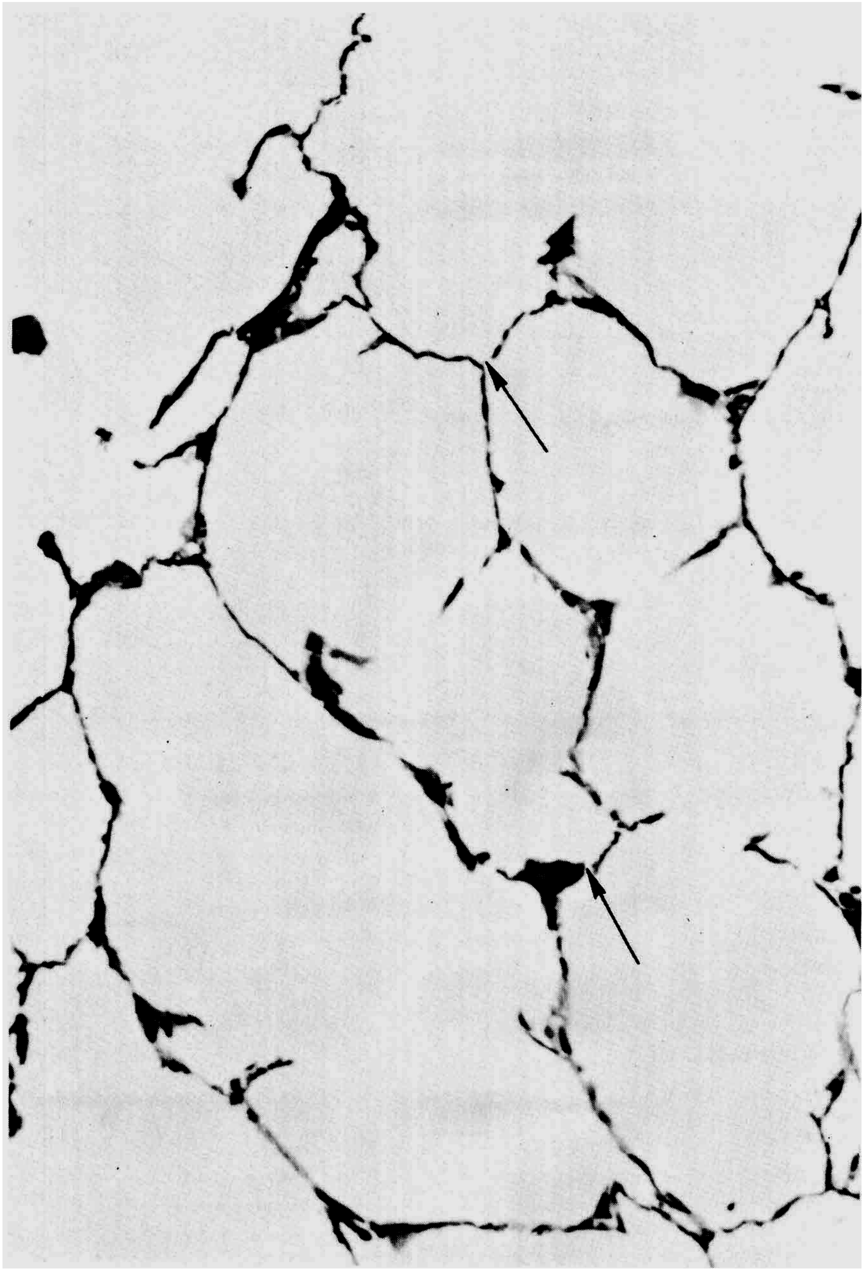

FIG. 2. Paraffin section from the left lung of a dog treated with papain. Note broken alveolar septa (arrow) and increased size of airspaces compared to Fig. 1. Hematoxylin and eosin. ×235.

sol was administered to the animals by intermittent positive pressure supplied by a Bird–Mark VII respirator set at a cycling pressure of 15 cm H_2O and powered by compressed air. This procedure was repeated two more times at 10-day intervals. Ten days following the third treatment a catheter was placed in the bronchial tree via the trachea with fluoroscopic guidance and 2.5 ml of the same papain solution was instilled. A Harvard respirator and an oxygen source were kept available to provide support for animals with transient respiratory failure. Two weeks after completion of the treatment the dogs were sacrificed with an overdose of sodium pentobarbital. The left lungs were inflated and fixed by intrabronchial instillation with 10% buffered formalin under 20 cm water pressure and sections cut for morphometric studies. Paraffin sections stained with hematoxylin and eosin or Verhoeff's elastic fiber stain were examined by light microscopy and the degree of emphysema was estimated by the difference in mean linear intercept between lungs from control and papain treated animals (18). The right lungs of four treated and four control dogs were freed of large blood vessels and major airways, homogenized and extracted with sodium chloride and butanol-acetone to isolate total connective tissue, an aliquot of which was further extracted with 0.1 *N* NaOH (19) to obtain purified elastin. Both the connective tissue and the elastin preparations were hydrolyzed in 6 *N* HCl and their amino acid composition determined on a Beckman 120C amino acid analyzer. As described in greater detail elsewhere (16, 17), mean elastin proportions were calculated by dividing the concentration of desmosine and isodesmosine in residues per 1000 in the crude connective tissue by the concentration of desmosine and isodesmosine in residues per 1000 in purified elastin from the same specimen. This ratio times 100 equals the percentage elastin in the connective tissue of the lung parenchyma. This method is preferred over gravimetric procedures because it is independent of variations in the weight of the dissected tissue after removal of large airways and blood vessels and can be used when entire lungs are not available, i.e., in large animals and humans.

Results. When the chest cavities were opened the lungs of the treated animals were found to be partially inflated with a few subpleural bullae 3–6 mm in diameter whereas the lungs of the control dogs were completely collapsed and lacked subpleural bullae. Microscopic examination of sections from the treated animals revealed enlarged air spaces and ruptured alveolar septa. Some regional variations were observed in that changes were always more severe in the caudal lobes than in the cranial lobes. Figures 1 and 2 are photomicrographs of lung sections of a control dog and a typical example of a treated animal. Morphometric data presented in Table I indicate that the mean linear intercept in the lungs of dogs treated with papain is significantly increased ($P < 0.01$).

Table II shows the amino acid analyses of total connective tissue isolated from the parenchyma of four control and four treated dogs. Mean elastin proportions calculated from the desmosine and isodesmosine content in the crude connective tissue and purified elastin derived from the same specimen were 14.42% ± SD 3.55 in the control dogs and 4.85% ± SD 1.84 in the animals with papain induced emphysema. The difference is highly significant ($P < 0.01$ by Student's *t* test) as shown in Fig. 3. Values obtained for the sum of desmosine and isodesmosine are reproducible to ± 8%. Table III shows the amino acid composition of the purified elastin indicating that the elastin from emphysematous lungs does not differ significantly from the elastin of normal dog lungs.

Two additional dogs were allowed to recover for 3 months before they were sacrificed and subjected to analysis. In these animals lung elastin proportions were no longer significantly different from those in normal dog lungs (mean 17.12% ± SD 2.2).

Discussion. The total amounts of papain administered to the dogs in this study exceed doses given to animals by other investigators reporting similar experiments. Kilburn *et al.* (3) instilled a single dose of 1 mg papain/100 g body wt in hamsters and Goldring *et al.* (2) used 3% solutions in an aerosol technique, whereas the dogs in the present study received a total of 13–14 mg/100 g body wt and 16% aerosols in four doses. In addition to the higher dosage the combination of aerosol and intratracheal instillation may also produce a more severe

TABLE I. MEAN LINEAR INTERCEPTS OF THE LUNG FROM CONTROL AND PAPAIN TREATED DOGS

Group	Number of dogs	Mean linear intercept
Control	4	$8.1\ (\pm 1.3) \times 10^{-2}$ mm
Papain treated	6	$12.3\ (\pm 3.8) \times 10^{-2}$ mm

TABLE II. AMINO ACID COMPOSITION OF THE CRUDE CONNECTIVE TISSUE FRACTION OF LUNGS OF CONTROL AND PAPAIN TREATED DOGS

Amino acid	Control dogs (1)	(2)	(3)	(4)	Papain treated dogs (1)	(2)	(3)	(4)
Lysine	19.97	22.89	28.98	35.55	25.59	29.76	26.84	24.21
Histidine	9.00	8.88	10.34	11.32	9.33	10.17	9.63	9.27
Arginine	41.82	43.31	41.90	46.90	49.97	48.89	49.00	49.32
Hydroxyproline	89.93	80.01	64.74	59.45	98.91	82.20	88.99	96.85
Aspartic acid	58.69	55.84	56.75	72.76	59.92	64.70	62.57	58.34
Threonine	24.28	25.27	27.78	34.90	23.31	27.33	25.57	23.27
Serine	41.26	40.10	39.43	48.58	42.40	44.98	43.58	42.07
Glutamic acid	83.36	80.76	72.76	94.14	87.13	89.49	85.38	84.02
Proline	105.02	104.82	95.34	92.64	104.56	96.43	99.63	106.28
Glycine	297.47	283.37	295.08	231.91	295.25	267.73	278.11	302.05
Alanine	91.02	103.13	111.12	94.23	81.87	94.56	97.22	84.13
Valine	29.72	36.72	38.91	38.51	22.50	27.73	35.95	23.53
Isoleucine	18.22	29.41	21.04	24.93	16.21	19.21	17.68	16.17
Leucine	36.59	39.92	44.97	51.80	34.56	41.19	37.75	32.73
Tyrosine	9.49	11.87	13.85	16.95	6.69	9.30	8.68	7.27
Phenylalanine	17.54	29.16	21.17	22.54	16.61	26.69	18.44	16.07
Isodesmosine	0.11	0.17	0.16	0.12	0.05	0.04	0.06	0.04
Desmosine	0.15	0.23	0.23	0.18	0.07	0.04	0.04	0.06
Hydroxylysine	9.25	8.49	5.85	3.18	10.42	8.98	9.03	10.08
Cysteic acid	1.43	5.40	2.51	4.94	3.22	2.92	0.97	4.31
Methionine sulfoxide	1.95	6.83	7.11	4.32	7.21	8.61	7.25	4.90
Cystine	4.93	1.49	—	4.23	1.93	1.92	5.53	1.61
Methionine	8.60	1.93	—	5.93	2.30	1.80	2.12	3.46
NP/P ratio[a]	2.72	2.77	2.88	2.05	2.39	2.25	2.38	2.51
% elastin	10.97	16.26	18.48	11.95	7.50	3.23	4.39	4.29

Note. Results expressed in amino acid residues/1000 residues.
[a] Nonpolar/polar amino acid ratio.

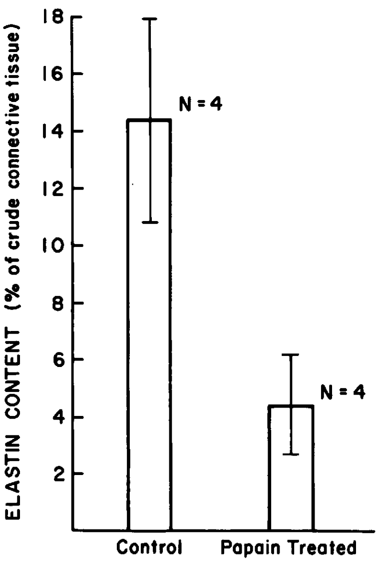

FIG. 3. Effect of intratracheal administration of papain on elastin proportions of canine pulmonary connective tissue.

form of emphysema than either route alone. The repeated administration of the enzyme mimics more closely the repeated insult sustained in human subjects than single doses and counteracts the tendency to regeneration of elastin in the early stages of the disease (14). While elastin proportions in lungs of animals with papain induced emphysema analyzed 3 months after the termination of the insult were not significantly different from those of normal dogs, 2 weeks after administration of the last dose significant diminution of the proportions of elastin could be demonstrated in all the animals. These results agree with previously reported data of Kuhn *et al.* (14) that lung elastin decreases at early stages of enzyme induced emphysema and support the concept that elastin content could be low or normal depending on the nature of the insult and the period elapsed after the termination of the insult until the measure-

TABLE III. AMINO ACID COMPOSITION OF THE PULMONARY ELASTIN FRACTION IN CONTROL AND PAPAIN TREATED DOGS

	Control dogs				Papain treated dogs			
Amino acid	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)
Lysine	3.74	4.01	4.05	5.04	5.13	4.77	3.74	3.87
Histidine	0.75	1.30	1.33	1.20	1.35	1.37	0.72	0.86
Arginine	4.87	6.27	5.93	5.90	8.24	6.19	5.46	6.24
Hydroxyproline	10.72	14.28	11.99	13.86	12.36	11.05	13.26	15.26
Aspartic acid	3.10	4.45	5.28	4.86	6.98	5.88	4.60	4.84
Threonine	11.30	14.41	13.99	12.27	14.97	13.79	12.04	14.89
Serine	9.16	10.73	9.55	11.52	13.46	11.59	9.83	10.90
Glutamic acid	15.74	18.74	16.49	19.27	20.10	17.86	16.32	18.25
Proline	112.75	115.11	107.78	116.48	109.51	107.59	107.53	114.21
Glycine	348.88	347.63	372.36	345.87	351.50	380.04	381.20	345.84
Alanine	267.64	233.17	237.00	237.20	247.88	240.72	245.99	233.38
Valine	94.34	101.91	92.80	101.08	95.63	88.76	89.50	103.91
Isoleucine	24.17	26.12	24.51	25.14	24.06	23.29	22.90	26.12
Leucine	43.53	49.36	45.94	48.24	42.90	42.34	42.16	48.11
Tyrosine	23.71	26.45	25.32	25.40	21.87	20.42	20.38	26.72
Phenylalanine	23.27	23.68	23.54	23.57	22.46	22.23	22.23	24.27
Isodesmosine	1.04	1.02	0.85	1.03	0.67	0.93	0.90	0.97
Desmosine	1.33	1.44	1.26	1.48	0.93	1.24	1.22	1.36
Hydroxylysine	0	0	0	0	0	0	0	0
Cysteic acid	0	0	0	0	0	0	0	0
Methionine sulfoxide	0	0	0	0	0	0	0	0
Cystine	0	0	0	0	0	0	0	0
Methionine	0	0	0	0	0	0	0	0
NP/P ratio[a]	31.61	25.11	26.62	24.05	20.83	24.47	28.84	25.59

Note. Results expressed in amino acid residues/1000 residues.
[a] Nonpolar/polar amino acid ratio.

ments are taken. The findings are also consistent with previous observations in human subjects with emphysema (17) and in rats with emphysema induced by intravenous injections of pancreatic elastase (20) and other data (15) indicating that pulmonary elastin is destroyed in emphysema. In two of the human subjects (17) where multiple samples were taken and assayed individually for elastin content, loss of elastin paralleled the severity of the disease as determined by mean linear intercept and by morphological observation.

The mechanism of elastin destruction in papain induced emphysema is not clear. The papain used has very low, though measurable, elastolytic activity determined by the digestion of fibrous elastin (21) on Alphasin plates (Elastin Products, St. Louis, Mo.) and by orcein–elastin tests. The value calculated from calibration curves established by parallel assays of pancreatic elastase of known activity against the same substrates was 0.087 units/mg. Although the doses administered were high, the total units ($1600 \times 0.087 = 139.2$) are well below those needed to induce emphysema of similar severity with elastase. Kaplan *et al.* (4) and Hayes *et al.* (5) instilled 12–25 units/100 g body wt compared to approximately 1 unit/100 g for 13-kg dogs. It has been suggested (22) that a factor in papain effects the release of elastolytic activity from polymorphonuclear leukocytes or alveolar macrophages. Preliminary experiments in our laboratory indicate that papain can stimulate or activate macrophages. In addition, the inflammation associated with papain induced emphysema may attract leukocytes to the area and stimulate elastolytic breakdown by these cells. The ability of papain to destroy α_1 antiprotease activity (23) may also play a role. Further work is required to clarify the mechanism by which the elastin is degraded.

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