THE ISOLATION OF ELASTIC TISSUE FROM LUNG*

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Rather drastic methods are commonly used to isolate elastic fibers from tissues; heating the tissue in 0.1 N NaOH (Lowry et. at., 1941) or autoclaving in water (Partridge, Davis and Adair, 1955) or dilute organic acid (Hall 1955) leaves a residue which is called elastin. As the name implies, the product so isolated undoubtedly represents partially degraded elastic fibers. In an attempt to isolate elastic fibers in a form more closely resembling that of the native state we have used digestion by trypsin and collagenase to remove extraneous protein. This report shows the difference in amino acid composition between elastin prepared by this procedure and that prepared by heating lung connective tissue in 0.1 N NaOH.

EXPERIMENTAL. Preparation of crude connective tissue: Fresh dog lung was dissected free of the major bronchi and blood vessels and blended with water. After collecting the connective tissue and cellular debri by centrifuging the suspension at low speed, the residue was washed with water and then stirred overnight in 1 M NaCl. The insoluble material was again collected, washed once with 1 M NaCl, three times with water and finally suspended in water. After freezing the mixture, the fibrous mass was dried in vacuo. Blending and washing was performed at approximately 5° C.

Lipid was removed from the dried tissue by two extractions with butanol at 0° C followed by three extractions with acetone at -10° C.

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After the last acetone extraction the tissue powder was dried by rubbing on sheets of filter paper. The product is called crude connective tissue (CCT).

PREPARATION OF ELASTIN. Crude connective tissue passing through a 70 mesh screen was subjected to alternate digestion by trypsin and collagenase.

Trypsin digestion: 1 gr CCT; 50 ml 0.1 M phosphate buffer, pH 7.0; 5 mg trypsin.

Collagenase digestion: Water washed residue from trypsin digest; 50 ml 0.01 M $CaCl_2$-0.05 M Tris buffer, pH 7.4; 2.5 mg collagenase. Incubation time was 2.5 hours at 37° C for each digestion. The elastin preparation described in this paper was subjected to a trypsin, collagenase, trypsin digestion sequence and is called enzyme elastin (EE).

Base elastin (BE) was prepared by heating 1 gr CCT in 25 ml 0.1 N NaOH at 100° C for 45 minutes (Lansing et. at., 1952).

RESULTS AND COMMENTS. Values for the amino acid composition of various elastin preparations are given in Table I. E-elastin differs significantly from B-elastin in the content of all amino acids except isoleucine, leucine, tyrosine and phenylalanine. The differences are particularly striking with respect to threonine, serine, and acidic and basic amino acids. B-elastin threonine is 0.59 that of E-elastin while the ratio of aspartic acid values of the two preparations is 0.14. Small differences in serine, threonine and arginine values might be expected due to the known destruction of these amino acids in protein on exposure to alkali. The large variation shown here however, led us to believe that a component(s) of the elastin fiber rich in these amino acids was lost on exposure to alkali. In order to test this hypothesis E-elastin was autoclaved in water at 15 lbs. pressure.* This treatment brought into solution about 36% of the total nitrogen. Amino acid compositions of the peptides (protein) (EAS) in the supernatant fluid and of the residue (EAR) remaining

* Elastins were autoclaved rather than heated in alkali to avoid destruction of sensitive amino acids.

Table I

Amino acid composition of elastin in N as % of Total N*

AMINO ACID	EE**	BE	EAR	EAS
Hydroxyproline	1.92	1.31	1.39	3.33
Aspartic Acid	2.32	.32	.67	5.08
Threonine	2.16	1.27	2.09	2.36
Serine	2.34	.85	1.30	3.56
Glutamic Acid	3.29	1.39	1.88	5.72
Proline	7.89	9.95	9.65	5.50
Glycine	24.7	32.4	31.6	15.0
Alanine	14.1	21.0	20.6	5.15
½ Cystine				.97
Valine	6.41	8.69	8.98	3.18
Methionine	.41			.46
Isoleucine	2.43	2.47	2.67	2.32
Leucine	4.16	4.24	4.56	3.87
Tyrosine	2.14	2.44	2.53	1.24
Phenylalanine	2.07	2.01	2.27	1.97
Lysine	2.00	.68	.84	3.95
Histidine	1.31	.32	.54	2.56
Ammonia	4.33	2.51	2.87	8.46
Arginine	6.60	2.29	3.06	12.2
Recovery %	90.6	94.1	97.5	86.9

* All values except those for BE (column 2) represent the average of analyses of two different preparations derived from the same CCT. The actual values obtained differ from average by not more than ±5% with the exception of methionine and cystine values which were within ±10%. Since the values are not definitive for elastic tissue and are given to illustrate major differences between preparations no correction for losses of amino acids on hydrolysis have been made.

** Collagen contamination is less than 6%. Digestion of EE with elastase brings into solution 94.5% of the total nitrogen. Elastase does not attack collagen.

after autoclaving are given in columns 3 and 4, Table I. The soluble peptides (protein) are rich in precisely those amino acids which show the largest decrease in going from E-elastin to B-elastin. Furthermore, the composition of the autoclaved residue closely resembles that of B-elastin. It is obvious that the material made soluble by autoclaving E-elastin cannot be collagen since the hydroxyproline and glycine content is much too low and the content of tyrosine is too high (see footnote to Table I). The soluble peptides (protein), in fact, are very similar in amino acid composition to contaminants of gelatin isolated by Maron (1958) and Leach (1960) and may be related to a tyrosine rich material with an apparent high turnover rate found in rabbit skin gelatin by Harkness, et. al., (1954).

Acid hydrolysis of EAS leads to pronounced humin formation and Warren's (1959) thiobarbituric acid assay gives color equivalent to 1% sialic acid, indicating the presence of carbohydrate in this soluble component(s). These facts support the observations (Hall, 1952; Banga and Schuler, 1953; Loeven, 1960) that a carbohydrate-protein complex is associated with elastic tissue.

The use of enzymes in the isolation procedure neither guarantees that the product is only elastic tissue nor precludes degradation of the native elastic fiber. While the pronounced specificity of collagenase makes it unlikely that this enzyme will attack native elastic fibers (see Mandl 1961), it is not at all certain that trypsin or a proteinase contaminating the collagenase does not remove some peptides from these fibers. The fact remains that the use of enzymes to digest extraneous proteins results in an elastin preparation which contains less than 6% collagen, differs markedly in amino acid composition from elastin prepared by heating the tissue in NaOH, and can be brought into solution almost completely by the enzyme elastase.

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