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Chemical Compositions of Elastins Isolated from Aortas and Pulmonary Tissues of Humans of Different Ages

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1. Elastins were isolated from the visceral pleuras and parenchymas of lungs of humans of different ages. 2. The elastin content of pleuras increased whereas that of parenchymas remained constant with increasing age. 3. The amino acid compositions and carbohydrate contents of elastins isolated from both pulmonary tissues changed in the same way with increasing age of the subjects. These changes were similar to those observed in elastins isolated from the aorta. 4. Similar glycoproteins were isolated from pleuras and aortas, and were more difficult to extract from the elastins of older subjects. Contamination with these glycoproteins was responsible for the changes in composition of elastin, as the age of the tissue from which it was extracted increased. 5. The amount of the cross-linking amino acids desmosine and isodesmosine was lower in elastins isolated from both aorta and pulmonary tissues of senile subjects than those from younger subjects.

Several theories have been proposed to explain the aging process in animals. One theory implicates connective tissues and supposes that changes occur during aging in the chemical and physical properties of the intercellular fibrous proteins, collagen and elastin. Such changes are thought to lead to an impairment in function of tissues. A common property of aortas and lungs from old subjects is a loss of elasticity. The lung is a highly elastic organ, most of the elastin being concentrated in the visceral pleura and parenchyma regions (John & Thomas, 1971). The aorta is another tissue rich in elastic fibres. The loss in elasticity could be due to a decrease in the amount of elastin in the tissues. However, the evidence is against this because with advancing age the elastin content in aortas either remains constant or increases slightly (Lansing, 1954) and in lungs the content actually increases (Pierce & Hocott, 1960). It seems more probable that changes in elasticity are due to changes in the elastic tissue itself. Studies have been made on the changes, with age, in the amino acid compositions of elastins purified from human aortas (Lansing, 1954) and elastins purified from peripheral portions of human lungs (FitzPatrick & Hospelhorn, 1962, 1965). A popular concept of aging is that the polypeptide chains in elastin, and also collagen, become excessively cross-linked in older subjects. There is evidence that the elastic fibres in animals are produced by an aggregation of soluble protein subunits, which are made insoluble by being covalently cross-linked to each other (Sandberg *et al.*, 1969). The cross-links have been identified as desmosine and isodesmosine (Thomas *et al.*, 1963) and lysinonorleucine (Franzblau *et al.*, 1965), and are produced by chemical interactions between certain lysyl residues in the polypeptide chains. The production of these cross-links is a part of the normal process of maturation of elastic fibres and should not be confused with the process of senescence. Elastin is synthesized at a rapid rate by young animals but at a much lower rate by adult animals. Apart from replacement of elastic tissue destroyed by disease processes and the mechanical effects of wear and tear it is believed that once mature elastic fibres are laid down they are there for life. Consequently the fibres are exposed for long periods to conditions which proteins with greater turnover rates would not encounter. These conditions may produce the changes that are attributed to aging and may lead to an increase in the number of covalent cross-links between peptide chains, which could either be the normal ones or abnormal ones. The yellow colour and fluorescence of elastic fibres increases with age (LaBella & Lindsay, 1963; Blomfield & Farrar, 1969). It has been suggested that the unidentified fluorescent substances are involved in the cross-linking of peptide chains in elastin. Another age-associated change that has been studied is the calcification of elastic tissue. The concentration of hydroxyapatite crystals within the elastic fibres in arteries has been shown to increase with age (Weissman & Weissman, 1960). However, there is little sign of calcification in the elastic fibres in normal lungs at any age.

In the present work a detailed study has been made of the changes in the chemical structures of

elastins purified from pulmonary tissues and from aortas extracted from very young to very old human subjects.

Experimental

Isolation procedures

Purification of elastin by using hot alkali. Samples of aorta, visceral pleura and parenchyma from lungs that were free of any observable diseases were obtained at autopsy from subjects of different ages. After removal of all adhering tissue, the samples were washed with water, freeze-dried, weighed, mixed with solid CO_2 and ground into a fine powder in a hammer mill. The powder was suspended in 1M-NaCl and homogenized in a VirTis 45 homogenizer operated at top speed for 3min. The fine creamy suspension was shaken overnight at 5°C and the insoluble material was recovered by centrifugation at 2075*g* for 20min. Extraction with saline was repeated twice more and the residue was washed with water until salt-free and was then defatted by suspending it in ethanol, then treating it overnight with chloroform–methanol (2:1, v/v). After separation by filtration through a sintered glass under suction the preparation was washed successively with acetone and ether and then dried in a desiccator. Insoluble collagen and other contaminants were extracted from the powder by treatment with 0.1M-NaOH at 98°C for 45min by the procedure of Lansing *et al.* (1952). The residual elastin was washed well with hot water and dried with organic solvents.

Purification of elastin by using enzymes. The aorta and visceral pleura of a 63-year-old male subject were removed and extracted with NaCl and organic solvents as described above. Collagen was removed by a procedure based on that of Miller & Fullmer (1966) and involved suspension of the powders (1g) in 5M-guanidine hydrochloride (20ml) at pH7 and stirring the suspension overnight at room temperature. The suspension was centrifuged and the residue was extracted twice more with guanidine hydrochloride. The residue was washed with water, dispersed in 0.2M-tris–HCl buffer, pH7.4 (20ml), then collagenase (5mg of type III, fraction A, Sigma Chemical Co., St. Louis, Mo., U.S.A.) was added with a drop of toluene and the mixture was incubated at 38°C for 48h with stirring. The suspension was centrifuged, then the supernatant was discarded, and the residue was washed with water and dried with organic solvents. Part of the powder was chemically analysed and the remainder was suspended in 0.1M-NH_4HCO_3, pH8.2 (20ml), then trypsin (2mg of bovine pancreatic, Sigma Chemical Co., St. Louis, Mo., U.S.A.) was added and the mixture was incubated at 38°C for 4h with stirring. The suspension was centrifuged and the elastin was washed with water and dried with organic solvents.

Isolation of glycoproteins. The aortas and visceral pleuras were removed from two male subjects aged 24 and 63 years and extracted successively with 1M-NaCl, organic solvents, guanidine hydrochloride and collagenase as described above. The glycoproteins were then released from the insoluble materials by treatment with cold dilute alkali (see Barnes & Partridge, 1968) and then with trypsin. The insoluble material was first treated with 0.1M-NaOH for 30min at 1°C. The solubilized material was removed by centrifugation, and then dialysed against distilled water; the non-diffusible material was freeze-dried. The residue remaining after alkaline extraction was further extracted by suspending it in 0.1M-NH_4HCO_3, pH8.2, and incubating it with trypsin (2mg) with stirring for 4h. The solubilized material was recovered by centrifugation and the supernatant was concentrated on a rotary evaporator and freeze-dried.

Analytical methods

Fluorescence spectra of solubilized elastins. Elastins (about 20mg) purified from aortas of subjects of different ages were solubilized by adding 5ml of constant-boiling HCl and keeping the mixture in a boiling-water bath for 2h. The HCl was removed on an all-glass rotary evaporator and the hydrolysate was dissolved in sufficient 0.05M-phosphate buffer, pH7.4, to give a protein concentration of 1mg/ml; then the fluorescent properties were determined on a Hitachi Perkin–Elmer MPF 2A spectrofluorimeter. The activation/fluorescence maxima of the hydrolysates were determined by scanning the fluorescence spectra over the range 200–800nm. The instrument was calibrated against a standard of quinine sulphate (1μM in 0.1M-H_2SO_4) to give 40% transmission. The intensity of fluorescence of the hydrolysates was expressed in arbitrary units.

Determination of carbon in pleura elastins. Elastins (about 30mg) purified by hot alkaline extraction from visceral pleuras of subjects of different ages were weighed into small glass-stoppered tubes, 5ml of 5.7M-HCl was added and the mixture was hydrolysed in a boiling-water bath for 2h. The tubes were centrifuged, then the carbon residues were washed with water, dried in an oven and weighed.

Determination of hydroxyproline. The method of Stegemann & Stalder (1967) was used.

Determination of carbohydrate. The anthrone method of Yemm & Willis (1954) was used. The results were expressed as galactose units.

Chromatography of monosaccharides. Samples were hydrolysed with 0.5M-H_2SO_4 for 2h at 100°C in sealed tubes. The monosaccharides were identified by paper chromatography as described by Partridge & Elsden (1961).

Amino acid analyses. Elastin samples (10mg in

5ml of constant-boiling HCl) were hydrolysed at 110°C for 72h in evacuated glass tubes. Other protein samples were hydrolysed for 1 day. The amino acid analyses were done on a Locarte Autoanalyzer by the procedure of John & Thomas (1971). Cystine was determined by measuring cysteic acid by Moore's (1963) method.

Results

Changes caused by age

Effect of age on the content and chemical composition of elastins in visceral pleuras of lungs. Elastins were isolated by hot alkaline extractions of salt-extracted and defatted visceral pleuras from lungs of subjects of different ages. Fig. 1 shows that the contents of elastins in dried pleuras increased from about 8% in young subjects to about 16% in very old subjects. At the same time the ratio of collagen to elastin decreased (Fig. 2), indicating that the collagen contents in the pleuras remained reasonably constant with increasing age. The chemical compositions of the elastins at different ages are shown in Table 1. Most of the N (96%) was recoverable as amino acid N. Apart from a tendency for the amounts of aspartic acid, glutamic acid, lysine and arginine to be higher after the second decade the compositions of the remaining amino acids were very much the same up to about the fifth decade. After that time more significant changes were seen in the analyses, especially in the very old elastins. The amounts of aspartic acid and glutamic acid increased significantly and the amounts of threonine, serine, lysine and arginine were higher whereas those of glycine, alanine and valine tended to be lower. Also changes were seen in the content of the cross-linking amino acids: the amount of desmosine plus isodesmosine stayed reasonably constant at 2.6 residues/1000 amino acid residues in elastins isolated from subjects aged up to about 40 years and then decreased gradually to a value of about 1.7 in the tissues from very old subjects. In the case of the other cross-linking amino acid, lysinonorleucine, there was a tendency for the values to be higher in the very old (1.8 residues/1000 amino acid residues) compared with the young (about 1.4). All the elastins contained small amounts of carbohydrate, the content increasing from about 0.2% (g/100g of elastin) to about 0.4% in the elastins from old subjects. The purified elastins were white in colour in the young, grey in middle-aged subjects and black in the very old. This was due to the presence of deposits of carbon, the amount being measurable after the third decade and then increasing to about 4% of the dried elastins.

Effect of age on the content and chemical composition of elastins in parenchyma of lungs. The elastins were purified by hot alkaline extraction from portions of parenchyma obtained from the lungs of

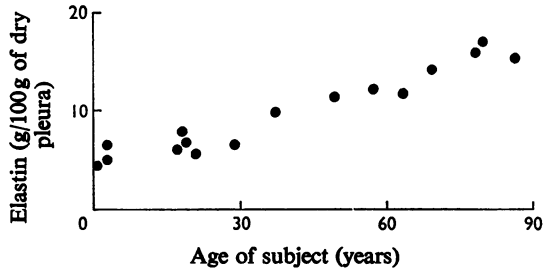

Fig. 1. *Elastin content of visceral pleuras isolated from subjects of different ages*

Tissues were extracted with 1M-NaCl, organic solvents and 0.1M-NaOH (40min at 98°C); the amount of insoluble elastin is expressed as g/100g of dried pleura.

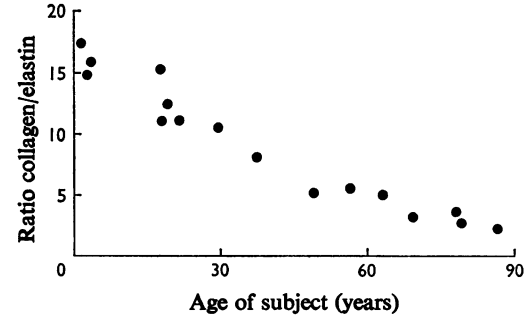

Fig. 2. *Ratio of collagen to elastin in visceral pleuras from subjects of different ages*

Experimental details are given in the text.

subjects of different ages. Unlike the visceral pleura, the content of elastin in the parenchyma did not seem to increase with increasing age, the amount remaining constant at about 25% of dried parenchyma. However, like the elastins isolated from pleuras the content of amino acids and the cross-linking amino acids of the parenchymal elastins changed in the same way (Table 2).

Effect of age on the chemical compositions of elastins isolated from aortas. The amino acid compositions of elastins prepared by hot alkaline extraction of aortas from subjects of different ages changed with increasing age in the same way as elastins isolated from pulmonary tissues (Table 3). Alterations became apparent only after the third decade, and then the amount of aspartic acid, glutamic acid, lysine and arginine increased gradually with increasing age. In the very old the average contents of glycine, alanine and valine tended to be lower than

Table 1. *Amino acid compositions of elastins purified from visceral pleuras of subjects of different ages*

Elastins were purified by hot alkaline extraction. Results are expressed in amino acid residues/1000 residues.

Age (years) ...	0.25	1.42	2	17	18	19	21	29	37	49	57	69	78	79	86
Sex ...	M	M	M	F	M	F	M	M	M	F	M	M	M	F	M
Hyp	10.1	9.1	12.1	9.5	12.0	11.1	10.1	11.9	11.5	11.0	11.0	11.1	12.7	11.4	12.5
Asp	4.5	2.9	3.0	3.8	5.5	5.0	3.7	5.0	7.3	6.7	6.4	8.0	8.1	10.5	10.3
Thr	9.2	8.2	9.2	7.0	8.7	9.3	7.8	9.4	9.9	9.7	8.3	10.3	10.4	9.5	10.7
Ser	5.1	5.8	6.0	4.5	4.6	6.2	5.8	5.6	4.7	5.5	6.7	5.8	5.9	7.7	7.8
Glu	16.8	14.0	16.5	17.2	18.1	17.1	17.8	19.1	21.4	21.7	21.6	23.1	24.0	26.3	26.6
Pro	120.0	124.0	123.0	121.0	125.0	116.0	118.9	117.0	118.0	119.0	113.0	119.0	117.2	117.0	119.0
Gly	307.0	310.0	304.0	313.0	306.0	314.0	314.0	313.0	308.0	311.0	305.0	297.0	299.0	296.0	297.0
Ala	239.0	239.0	232.0	241.0	236.0	241.0	242.0	233.0	228.0	228.0	235.0	233.0	231.0	231.0	227.0
Val	142.0	148.0	142.0	147.0	141.0	140.0	142.0	143.0	141.0	139.0	141.0	140.0	136.0	134.0	135.0
Ile	25.2	25.0	28.8	24.6	25.7	25.8	24.7	25.5	25.8	25.8	24.0	24.4	25.0	24.8	25.0
Leu	62.9	58.3	60.2	59.0	59.8	58.0	59.3	59.0	60.0	58.5	62.4	62.3	62.8	58.5	56.5
Tyr	16.9	18.0	22.2	16.9	18.5	18.9	17.3	19.8	21.5	21.4	20.0	21.8	21.6	23.4	22.2
Phe	25.0	22.3	25.0	19.5	21.6	20.3	21.8	23.1	23.0	21.9	22.6	22.6	23.1	23.5	23.4
Isodesmosine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9	0.7	0.8	0.7	0.7
Desmosine	1.7	1.7	1.8	1.6	1.6	1.6	1.6	1.6	1.5	1.4	1.3	1.1	1.0	1.0	1.0
Lysinonorleucine	1.4	1.4	1.4	1.4	1.5	1.5	1.4	1.5	1.6	1.8	1.7	1.9	1.9	1.8	1.8
Orn	1.1	0.8	0.9	1.6	1.3	1.3	1.3	1.3	2.0	2.2	3.4	2.5	2.2	2.2	3.0
Lys	6.2	5.6	5.9	5.4	6.4	5.7	5.7	6.1	7.5	8.4	8.2	8.2	9.9	10.3	10.8
Arg	6.1	4.9	5.4	5.5	6.3	5.8	4.5	4.9	7.1	6.8	7.1	8.0	8.1	10.2	10.0
Total	1000.4	1000.0	1000.4	1000.5	1000.5	999.6	1000.7	1000.8	1000.8	1000.7	999.6	1000.8	1000.7	999.8	1000.3
Carbohydrate (g/100g of elastin)	0.21	0.22	0.20	0.23	0.25	0.23	0.24	0.23	0.26	0.27	0.32	0.36	0.39	0.42	0.42
Carbon (g/100g of elastin)	—	—	—	—	—	—	—	—	1.0	3.5	3.6	3.5	3.0	3.7	3.9

Table 2. *Amino acid compositions of elastins purified from the parenchyma of subjects of different ages*

Elastins were purified by hot alkaline extraction. Results are expressed in amino acid residues/1000 residues.

Age (years) ...	17	21	50	71
Sex ...	M	M	M	F
Hyp	11.0	10.0	11.5	11.0
Asp	5.1	6.4	8.5	10.2
Thr	8.3	9.4	9.0	10.2
Ser	7.2	7.2	7.3	7.1
Glu	20.3	22.4	26.4	28.8
Pro	113.0	121.5	116.0	115.0
Gly	307.0	306.0	303.0	296.0
Ala	240.0	229.0	226.0	222.0
Val	140.5	135.0	135.0	130.0
Ile	25.0	28.2	25.8	26.4
Leu	58.5	62.4	60.0	60.6
Tyr	21.2	20.0	23.4	26.0
Phe	23.2	23.8	24.5	26.6
Isodesmosine	1.0	1.1	0.9	0.8
Desmosine	1.6	1.6	1.4	1.0
Lysinonorleucine	1.5	1.7	1.8	2.0
Orn	1.5	1.6	2.0	3.1
Lys	6.9	7.0	9.5	13.5
Arg	7.9	6.2	8.3	10.4
Total	1000.7	1000.5	1000.3	1000.7

the younger elastins. The content of desmosine plus isodesmosine was higher in aorta than in pulmonary tissues of the same age and remained at 3.0 residues/1000 amino acid residues up to about the sixth decade. After this time the content of desmosine plus isodesmosine decreased to about 2.2 residues in the elastins from senile subjects. The carbohydrate contents of the aortic elastins were slightly lower than the pulmonary elastins but the content increased with age. All the aortic elastins had a white colour and did not contain carbon deposits.

Effect of age on the fluorescence spectra of elastins purified from human aortas and human visceral pleuras. Elastins solubilized with acid or proteinases fluoresce in ultraviolet light. Three fluorescent substances are present in elastin; two are unidentified, with activation/fluorescence maxima at 250/370nm (substance X) and 336/393nm (substance Y); the third spectrum (284/308nm) is identical with that of tyrosine. Elastins were purified with hot alkali from the aortas and pleuras of human subjects of different ages and then solubilized by heating with conc. HCl. The intensity of fluorescence of substances X and Y was determined on the elastins of different ages and the results for aortas are shown in Fig. 3. The intensity of fluorescence of substance X remained constant whereas that of substance Y increased. The results for pleuras were very similar.

Purification of elastin by enzymic digestion of aorta and visceral pleura

The tissues from a 63-year-old subject were extracted successively with 1M-NaCl, organic solvents and 5M-guanidine hydrochloride. Collagen was solubilized by incubation with collagenase. The residue was analysed for amino acids. Table 4 shows that the compositions of the residues isolated from both aorta and pleura are similar to that of elastin, but the concentrations of aspartic acid, threonine, serine, glutamic acid, methionine, lysine and arginine are higher whereas those of glycine, alanine and valine are lower. The desmosines and lysinonorleucine were present but their concentrations were lower than in elastin purified with hot alkali. This suggested that there was contamination with another protein. An attempt was made to solubilize this material by tryptic digestion. The insoluble material remaining was analysed for amino acids. The analysis, although more similar to that of elastin, was still not that of pure elastin. A further amount of contaminating protein was freed from the insoluble elastin by adding 0.1M-NaOH and keeping the mixture at 98°C for 40min. Enzymic digestion of the aorta and pleura of a 23-year-old subject also did not produce pure elastin. Prolonged incubation of elastin preparations caused hydrolysis of elastin as shown by analysis of the solubilized materials.

Table 3. *Amino acid compositions of elastins purified from aortas of subjects of different ages*

Elastins were purified by hot alkaline extraction. Results are expressed as amino acid residues/1000 residues

Age (years)	0.25	1.42	2.0	17	18	19	29	42	49	57	63	69	73	78	79	86
Sex	M	M	M	F	M	F	M	M	F	M	M	M	F	F	F	M
Hyp	11.0	10.6	10.0	9.1	8.4	9.5	9.0	10.3	9.9	11.1	11.0	10.6	11.0	11.1	12.2	11.4
Asp	2.9	2.6	2.6	2.9	3.9	3.3	3.0	6.4	7.5	5.6	6.5	6.5	10.2	8.8	10.5	9.9
Thr	6.6	9.1	8.4	7.5	8.8	8.1	7.8	9.3	8.6	8.2	8.5	8.9	10.1	10.0	8.3	10.6
Ser	3.4	6.7	5.2	5.0	5.6	4.0	5.2	5.3	5.5	6.7	5.7	5.8	7.7	7.2	7.7	8.0
Glu	14.8	15.0	15.5	14.5	16.7	16.4	17.8	20.6	20.9	21.0	21.5	21.9	27.7	26.6	27.8	26.2
Pro	122.5	126.0	127.0	128.2	125.5	128.0	124.5	123.0	120.0	122.0	117.0	122.5	116.0	120.3	125.0	123.0
Gly	309.0	304.0	301.0	318.0	311.0	313.0	313.0	303.0	317.0	301.0	308.0	302.0	299.0	304.0	296.0	303.0
Ala	237.0	237.0	237.0	236.0	239.0	241.0	240.0	237.0	227.0	228.0	227.0	231.0	225.0	223.0	226.0	224.0
Val	145.0	145.0	147.0	143.0	139.0	136.0	144.0	138.0	136.5	145.0	148.0	139.0	137.0	141.0	135.0	136.0
Ile	24.9	24.7	25.0	23.4	25.2	24.3	22.4	25.4	25.0	25.4	24.6	24.9	25.2	25.8	25.4	25.2
Leu	59.8	59.4	60.0	56.6	58.6	56.9	55.8	59.4	58.8	64.5	57.1	61.5	60.8	59.3	58.4	57.0
Tyr	19.7	21.0	21.3	19.0	19.8	21.0	20.7	21.1	20.2	20.2	21.8	22.6	22.0	21.2	22.0	21.2
Phe	24.4	23.0	24.2	21.7	21.6	22.9	23.8	23.2	23.6	24.3	23.9	23.6	24.3	22.7	22.4	22.4
Isodesmosine	1.2	1.2	1.2	1.1	1.1	1.1	1.1	1.0	1.0	1.1	1.1	0.9	1.0	0.9	0.8	0.8
Desmosine	1.8	2.0	2.1	1.8	1.9	2.1	1.8	1.9	1.9	1.9	1.9	1.7	1.4	1.4	1.3	1.4
Lysinonorleucine	1.4	1.4	1.5	1.3	1.3	1.3	1.2	1.8	1.8	1.6	1.7	1.6	1.8	1.7	1.9	1.8
Orn	1.3	0.9	1.0	1.4	1.2	1.4	1.1	1.6	2.2	2.0	1.9	2.1	2.3	1.7	1.7	1.8
Lys	7.6	6.6	6.0	4.7	5.0	4.4	4.0	5.4	6.4	5.1	6.0	5.8	8.1	6.5	8.7	7.9
Arg	5.5	4.4	4.4	5.2	5.7	5.9	4.4	7.2	6.1	6.5	6.5	7.3	9.5	7.9	8.0	8.0
Total	999.8	1000.6	1000.4	1000.4	999.3	1000.6	1000.6	1000.9	999.9	1001.2	999.7	1000.2	1000.1	1001.1	999.1	999.6
Carbohydrate (g/100g of elastin)	0.11	0.11	0.10	0.13	0.16	0.11	0.14	0.20	0.21	0.22	0.22	0.25	0.27	0.30	0.33	0.32

Fig. 3. *Effect of age on the intensity of fluorescence of elastins purified from aortas of subjects*

o, Intensity of fluorescence of substance X; ●, intensity of fluorescence of substance Y.

Isolation of glycoproteins from aortas and visceral pleuras of subjects aged 24 years and 63 years

After successive treatments of aortas and pleuras with 1M-NaCl, organic solvents, guanidine hydrochloride and collagenase, the residues were extracted successively with 0.1M-NaOH for 30min at 1°C and then with trypsin. The amino acid compositions of the alkaline and tryptic solubilized materials are shown in Table 5. The materials solubilized by both procedures, from both aorta and pleura, are similar in amino acid contents irrespective of the age of the tissue. Also these proteins contain considerable amounts of carbohydrate (5%), consisting of fucose, galactose, mannose, *N*-acetylglucosamine, *N*-acetylgalactosamine and sialic acid. These glycoproteins are very different from elastin, the concentrations of aspartic acid, threonine, serine, lysine and arginine being much higher than that of elastin whereas those of glycine, alanine and valine are much lower. Also these glycoproteins contain cystine and methionine, which are only found in trace amounts in elastin. The absence of hydroxyproline and hydroxylysine indicated the absence of any contaminating collagen.

Discussion

The elastin content of human aortas remains constant or increases slightly after the second decade of life (Lansing, 1954). This is in contrast with human lungs, a distinct increase in elastin content throughout life being reported by several groups (Briscoe & Loring, 1958; Pierce & Hocott, 1960; Wright *et al.*, 1960). This increase in pulmonary elastin throughout adulthood seems to be confined to the visceral pleura,

Table 4. *Amino acid compositions of elastins purified with enzymes from the aorta and visceral pleura of a 63-year-old subject*

The tissues were first extracted with NaCl, organic solvents and guanidine hydrochloride and the residues were analysed after successive treatments with collagenase, trypsin and hot alkali. Results are expressed as amino acid residues/1000 residues.

	Aorta			Pleura		
Treatment ...	Collagenase	Collagenase and trypsin	0.1 M-NaOH at 98°C, 40 min	Collagenase	Collagenase and trypsin	0.1 M-NaOH at 98°C, 40 min
Hyp	9.0	10.5	11.0	7.0	9.0	11.0
Asp	21.8	9.3	6.5	30.6	12.9	7.2
Thr	19.1	16.0	8.5	23.4	16.3	9.3
Ser	13.7	10.5	5.7	22.6	14.1	6.3
Glu	36.6	24.7	21.5	50.0	27.3	23.3
Pro	113.5	124.7	117.0	76.7	121.0	116.0
Gly	271.0	293.0	308.0	258.6	292.0	302.0
Ala	207.0	221.8	227.0	206.9	215.0	234.0
Val	125.0	135.2	148.0	126.7	133.0	139.0
Met	3.9	Trace	Trace	5.5	Trace	Trace
Ile	29.3	26.3	24.6	28.9	25.8	24.2
Leu	63.3	59.8	57.1	69.7	61.0	62.3
Tyr	24.8	23.3	21.8	21.1	22.8	20.9
Phe	27.4	23.6	23.9	26.9	24.0	22.6
Isodesmosine	1.0	1.2	1.1	0.6	0.8	0.8
Desmosine	1.6	1.6	1.9	0.9	0.9	1.2
Lysinonorleucine	0.9	1.1	1.7	—	1.2	1.7
Orn	—	—	1.9	—	1.8	2.2
Lys	15.4	8.2	6.0	22.7	12.1	8.2
Arg	15.8	9.7	6.5	22.2	10.5	7.6
Total	1000.1	1000.5	999.7	1001.0	1000.5	999.8

the parenchymal content remaining reasonably constant.

John & Thomas (1971) found it more difficult to extract all soluble contaminants from the insoluble elastin in pulmonary tissues than from aortas. This was attributed to a difference in fibre structure between the two tissues. However, by employing a purification procedure that involved extraction in hot alkali, elastins of similar compositions could be obtained from aortas and pulmonary tissues. Elastins isolated from visceral pleuras and parenchymas of lungs from babies to 86-year-old human subjects showed little change in chemical composition until after the second decade. During middle age the elastins contained slightly more aspartic acid, glutamic acid and carbohydrate but slightly less of the cross-linking amino acids, desmosine and isodesmosine. Elastins isolated from very old subjects showed greater changes, the concentration of hydrophilic amino acids and carbohydrate being significantly higher than in elastins from young subjects. Also the amount of cross-linking in old elastins changed, as the content of desmosine and isodesmosine decreased by one-third whereas that of lysinonorleucine increased slightly. Apart from a tendency of the content of desmosine plus isodesmosine to remain constant during middle age, elastins isolated from aortas of human subjects exhibited the same pattern of change with advancing age. Lansing (1954) explained the changes in amino acid composition of aortic elastins isolated from senile subjects as being due to contamination with proteins containing high concentrations of hydrophilic amino acids. It was suggested that these contaminants were more difficult to extract from the elastic fibres of aortas from old subjects than from those of young ones. In the present study the pleuras of lungs isolated from both young and old subjects contained glycoproteins that accounted for 5% of the dried tissues. These glycoproteins were insoluble in saline and seemed to occur in two fractions, one fraction that could be solubilized with cold dilute alkali and trypsin, and a second that was very insoluble and required more drastic treatment, such as hot alkali. In the case of

Table 5. *Amino acid compositions of glycoproteins isolated from the aortas and visceral pleuras of subjects aged 24 years and 63 years*

After successive treatments of tissues with NaCl, organic solvents, guanidine hydrochloride and collagenase the residues were extracted with cold dilute NaOH followed by trypsin and the extracts analysed. Results are expressed as amino acid residues/1000 residues.

	Aorta				Pleura			
Age (years) ...	24		63		24		63	
Treatment ...	NaOH	Trypsin	NaOH	Trypsin	NaOH	Trypsin	NaOH	Trypsin
Asp	100.5	97.0	95.5	89.0	105.3	102.0	104.0	95.8
Thr	52.0	53.0	51.2	45.8	51.4	53.5	51.7	49.6
Ser	65.1	69.0	62.5	65.5	67.9	68.0	69.3	62.5
Glu	124.6	119.0	114.5	110.0	129.3	131.0	124.0	128.0
Pro	60.9	68.6	77.5	78.2	53.0	62.8	54.7	78.4
Gly	96.5	110.0	101.0	127.5	108.7	95.2	108.0	92.5
Ala	75.6	88.5	79.6	88.0	75.6	82.6	72.5	84.5
Cys	13.8	8.3	12.3	14.0	13.4	8.9	13.4	8.3
Val	67.3	64.0	67.2	69.8	69.8	60.8	69.8	66.3
Met	18.3	7.3	17.2	12.9	14.6	12.8	13.5	16.5
Ile	43.2	39.2	42.7	37.0	40.5	38.0	37.6	36.4
Leu	88.0	83.5	85.7	81.0	92.2	90.2	97.0	88.4
Tyr	33.5	25.2	35.5	28.4	25.3	23.9	24.1	22.7
Phe	40.1	33.2	42.6	34.6	38.1	34.7	37.8	32.0
His	21.0	21.4	20.2	16.6	22.6	19.6	20.9	22.3
Lys	49.7	63.0	50.0	50.7	45.8	66.6	49.4	62.4
Arg	50.0	50.7	46.4	51.2	46.3	49.9	52.9	54.1
Total	1000.1	1000.9	1001.6	1000.2	999.8	1000.5	1000.6	1000.7

the older elastins a small fraction of these glycoproteins seemed to be so intimately associated with the elastic fibres that it could only be removed at the risk of dissolving the elastin itself. The glycoproteins extracted from pleuras contained high concentrations of hydrophilic amino acids, especially aspartic acid and glutamic acid, which are found in low concentrations in elastin. It seems reasonable to assume that contamination with these glycoproteins is responsible for the difference in composition of elastins with advancing age. There are two possible explanations for the increasing association of glycoproteins with elastin with advancing age: the glycoproteins may become more insoluble as a result of increased disulphide cross-linking; or because of the low metabolic turnover of adult elastin, the glycoproteins are in contact with elastin for a long time and eventually become chemically bound to the elastin. Glycoproteins very similar in composition and properties to those of pulmonary tissues have been isolated from human aortas in the present work and by Barnes & Partridge (1968) and by Robert *et al.* (1970). Like pleural elastins, the change in composition of aortic elastin with advancing age could be due to increasing contamination with these glycoproteins. A number of reports have appeared indicating the presence, in a variety of connective tissues, of similar glycoproteins that seem to act as structural proteins in association with collagen and elastin (Wolff *et al.*, 1971).

Elastins isolated from the aortas and pulmonary tissues of senile human subjects contain about 30% less desmosine plus isodesmosine than those isolated from young subjects. The glycoproteins described above do not contain desmosines, hence their presence in elastin could lead to a lowering in the measured contents of desmosines in elastin preparations. By using the amount of aspartic acid and glutamic acid in elastins isolated from tissues of older subjects in excess of that from young subjects as a measure, the amount of contamination of glycoprotein in elastin preparations can be determined. In elastins isolated from tissues of middle-aged subjects the glycoprotein contamination was a small percentage and could have had little effect on the desmosine content. However, in the case of senile

subjects, the glycoprotein could account for 10% of the elastin preparation, causing a lowering in the content of desmosines by the same amount. This leaves a decrease of 20% in the content of the desmosines in elastins from senile subjects compared with elastins from young subjects, which could only be explained by assuming that the desmosines were destroyed in old age. Thomas *et al.* (1963) reported that desmosines were sensitive to oxidizing agents, the products of oxidation being aspartic acid, glutamic acid and lysine. However, the decrease in the concentrations of the desmosines observed in aortic and pulmonary elastins from old subjects is not enough to account for the increase in the contents of these oxidation product amino acids.

The only cross-links recovered in this survey are those that are stable to strong acid hydrolysis. Lent & Franzblau (1967) isolated the aldol produced by the condensation of the side-chain groups of two lysyl residues from elastin preparations. This compound could act as a cross-linking amino acid in elastin. No information is available on the effect of increasing age on the concentration of this cross-link in elastin. Also with increasing age elastins become more fluorescent and acquire a yellow colour. The materials responsible for this have not been identified and their effect on chemical cross-linking has not been elucidated.

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