tumor tissue. This increase we have termed the irradiation produced rise. In a series of patients the magnitude of the IPR in general has been found to increase during radiation thyroidectomy with the quantity of radioiodine retained by the thyroid. In a given patient the magnitude and incidence of the IPR decrease with successive doses as functioning thyroid tissue is progressively destroyed. Finally, when there is no longer any functioning tissue, as demonstrated by absence of I* uptake, the blood radioiodine concentration rapidly falls to very low levels and there is no IPR.

The blood of 21 patients, in various stages of therapy, was studied. For all of 7 radiation thyroidectomy doses, significant IPR's were found; in thyroidectomized patients with functioning metastases, IPR's were demonstrated in 8 of 33 doses. However, no such rise in blood iodine concentration was observed following four doses to thyroidectomized patients lacking demonstrable I* uptake. Since no such rise is found in the absence of functioning thyroid tissue, either neoplastic or non-neoplastic, the occurrence of an IPR in a thyroidectomized patient may, therefore, be considered evidence of the existence of viable functioning thyroid carcinoma metastases.

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Changes with Age in Amino Acid Composition of Arterial Elastin.* (18604)

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During a study of human arteriosclerosis it became evident that changes in the media of the aorta preceded the formation of intimal plaques(1,2). In particular, the elastic tissue of the tunica media, free of minerals in youth, became heavily calcified in adult and aging individuals.

Elasto-calcinosis, as the process of calcification of arterial elastic tissue has been termed recently(1), is accompanied by a number of alterations in the morphologic appearance and tinctorial reactions of elastic fibers. In the light of these differences, we have initiated a program to compare the chemical properties of young and old elastic fibers. The changes with age in the amino acid composition of human arterial elastin are now being reported.

Experimental. Analytical procedures. Elastin was prepared from human aortas (aortic elastin) and pulmonary arteries (pulmonary elastin) and calcium determinations were made on ashed samples by methods which have been described previously(3). The nitrogen contents were determined and the amino acid distributions were made by microbiological and paper chromatographic methods, the details of which have also been given previously(4). Several phosphate determinations on the ash from elastin gave a molar Ca:P ratio of approximately 3:2. For purposes of calculation it was, therefore, assumed

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that all of the calcium was present in the form of $Ca_3(PO_4)_2$.

Preparation of heavy and light fractions. It was found that a separation of fractions having differing specific gravities could be achieved by differential centrifugation of finely ground elastin (Wiley mill) in sucrose solution (sp. gr. 1.30). Most of the material in samples of aortic elastin from very young individuals floated on the solution after centrifugation, while most of that from old individuals settled to the bottom. A sample of the suitably washed light fraction (young light) was prepared from several young aortas and some of the heavy material (old heavy) was prepared from old aortas. Both of these materials were analyzed for calcium and the contents of 18 amino acids.

Results. Calcium and nitrogen contents. The calcium and nitrogen contents of samples of elastin obtained from human autopsy specimens are given in Table I. The calcium content of the young aortic elastin was low, while that from the older specimens was markedly elevated. Calcium accumulation did not take place with age in the pulmonary elastin. The nitrogen contents of all of the samples were similar when corrections were made for the calcium content.

Paper chromatographic findings. Two dimensional chromatograms were made of acid hydrolysates of 15 samples of elastin from aortas of people of different ages. In all instances samples containing 77 μg of nitrogen were placed on paper for chromatography. The chief amino acids found in the samples were glycine, alanine, valine, the leucines, and proline. Also detected, but in much smaller concentrations, were tyrosine, serine, arginine, and glutamic and aspartic acids. The most striking visual difference between the chromatograms of aortic elastins from young and old specimens was the increased intensity of the spots corresponding to aspartic and glutamic acids in the latter. These findings made it seem advisable to perform quantitative determinations of the contents of some of the amino acids in the elastin preparations. As will appear in these quantitative analyses, other amino acids such as arginine, histidine, and threonine also increased with age.

TABLE I. Amino Acids, Calcium, and Nitrogen Contents of Elastin from Aortas and Pulmonary Arteries of Young and Old Individuals.

Sample	Age	Aspartic acid, g N/100 g N	Glutamic acid, g N/100 g N	Glycine, g N/100 g N	Proline, g N/100 g N	Leucine, g N/100 g N	Isoleucine, g N/100 g N	Valine, g N/100 g N	Amide NH_3 g N/100 g N	g Ca/100 g	g N/100 g† (corrected)
Young aortas	15	.24	1.32	26.6	11.4	4.71	2.22	13.6	1.43	.17	15.90
	17	.32	1.67	25.8	11.1	4.76	2.31	12.3	1.43	.16	15.75
	19	.31	1.43	27.0	10.8	4.94	2.27	13.2	1.43	.18	15.82
	20	.38	1.63	26.8	11.3	4.84	2.38	12.9	—	.88	15.35
	Mean	.31	1.51	26.6	11.2	4.81	2.30	13.2	1.43	.35	15.71
Old aortas	55	.65	2.13	25.6	10.4	4.92	2.46	11.4	1.83	4.58	16.22
	59	1.35	2.70	24.0	9.4	5.01	2.43	11.4	2.30	4.74	15.50
	65	.68	2.16	24.8	11.3	4.59	2.44	11.9	1.76	6.03	15.19
	65	1.70	3.06	22.6	9.4	4.94	2.60	10.7	2.40	6.61	15.37
	74	1.15	2.33	25.2	10.7	4.73	2.21	12.0	2.22	5.14	15.32
	75	.90	2.52	23.8	10.2	4.60	2.47	11.4	2.02	8.47	15.75
	Mean	1.07	2.48	24.3	10.2	4.80	2.43	11.5	2.09	5.93	15.56
Pulmonary arteries	15	.68	1.84	30.4	11.5	4.68	2.24	12.2	1.51*	.13	16.25
	26	.66	1.99	31.3	10.7	4.96	2.28	13.6	(1.38-	.17	15.68
	77	.47	1.40	31.7	9.8	4.53	2.14	12.4	1.76)	.15	16.27
	92	.68	1.75	34.5	11.8	4.95	2.47	13.4		.64	15.78
	Mean	.62	1.75	32.0	11.0	4.78	2.28	12.9	1.51	.27	16.00

* These values were obtained from the analysis of 10 different samples of elastin prepared from pulmonary arteries ranging in age from 31 to 79 yr.
† Calculated on a Ca-free basis, assuming all of the Ca to be present as $Ca_3(PO_4)_2$.

Amino acid analyses. In Table I are shown the results of analyses for 7 amino acids. Highly significant increases in the contents of aspartic and glutamic acids were noted in the samples of old aortic elastin as compared to those of the young elastin, in confirmation of the chromatographic findings. There also were increases in the contents of amide nitrogen. However, there was a 4-fold increase in the excess of dicarboxylic acids over amide nitrogen, showing that the quantity of free carboxyl groups was increased significantly in the older specimens. The mean values for valine, proline, and glycine contents were somewhat lower in the older samples, while the leucine and isoleucine contents in the two groups were closely similar. In contrast to the findings for aortic elastin, the pulmonary elastin did not show increases in aspartic and glutamic acid contents with age. The other amino acids also did not change significantly. The contents of proline, leucine, isoleucine, and valine were virtually identical with those found in young aortic elastin. However, the contents of glycine and the dicarboxylic amino acids were higher in the pulmonary elastin. The quantity of free carboxyl groups was at least twice as great as that found in young aortic elastin, while the range showed some overlap with that of the old aortic elastin. In general, the values for the amino acids found in these human elastins were in the range of those previously reported for the aortic elastin of the pig, sheep and cattle(5) prepared by different methods.

Amino acids in young light and old heavy aortic elastins. In Table III are shown the contents of 18 amino acids in the light fraction of young aortic elastin and the heavy fraction of old aortic elastin prepared by suspension in sucrose in the manner described previously. In the case of the 7 amino acids which were determined on whole elastin (Table I), the differences observed between the young light and old heavy fractions were all similar to those found for the young and old whole elastin. The old heavy elastin showed increases in aspartic and glutamic acids and decreases in the contents of glycine, proline, and valine. There was also an increase in the number of free carboxyl groups in the old heavy sample. In addition, there was a decrease in the content of alanine. All of the other amino acids showed increases of varying degree in the old heavy elastin. Ap-

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TABLE II. Dicarboxylic Acid and Amide Contents of Elastin from Aortas and Pulmonary Arteries of Young and Old Individuals.

		Millimoles N/100 g total N		
Sample	Age	A Aspartic + glutamic	B amide	A-B
Young aortas	15	112	103	9
	17	142	103	39
	19	124	103	21
	Mean	126	103	23
Old aortas	55	199	130	69
	59	290	164	126
	63	203	126	77
	65	339	172	167
	74	248	158	90
	75	244	144	100
	Mean	254	149	105
Pulmonary arteries	15	180	108*	72
	26	189	108	81
	77	134	108	26
	92	174	108	66
	Mean	169		61

* See footnote * in Table I.

TABLE III. Amino Acid Composition of Young Light and Old Heavy Elastin.

Amino acid	Young "light"* g N per 100 g N	Old "heavy"† g N per 100 g N
Aspartic	0.38	1.11
Glutamic	1.83	3.01
Glycine	26.10	21.30
Proline	10.10	9.20
Leucine	4.52	4.76
Isoleucine	2.10	2.33
Valine	13.00	11.50
Alanine	23.18	21.58
Lysine	0.49	1.17
Arginine	1.78	4.35
Histidine	0.15	0.75
Cystine	0.06	0.10
Methionine	0.06	0.35
Phenylalanine	1.73	1.97
Tyrosine	1.45	1.76
Tryptophan	0.06	0.24
Serine	0.29	0.70
Threonine	0.65	1.13
Amide N	2.90	2.86
Total	90.83	90.17

* This preparation contained 1.14% calcium and 14.9% N.

† This preparation contained 6.39% Ca and 12.5% N.

proximately 90% of the nitrogen of both samples was accounted for by the analyses. The light fraction contained 1.14% calcium while the heavy fraction contained 6.39%.

Discussion. The data in this paper show that the calcific changes previously observed to occur with age in the medial elastic tissue of the human aorta(2) are accompanied by changes in the amino acid composition of the elastin. It is doubtful that the shift in amino acid distribution represents a change with time in the amino acid composition of a pure protein. The elastin studied may be a mixture of two or more proteins, and the changes observed may be related to differences in the ratios of the separate constituents How such a change can come about is not yet known. It is possible that the proteins laid down later in life may be different from those formed earlier because they are formed under the influence of fibroblasts which themselves have undergone age changes. Previous reports(3) from this laboratory have emphasized the fact that the pulmonary artery is characterized by a low rate of calcification of the medial elastic elements. This investigation brings forth evidence that no significant amino acid changes occur with age in pulmonary elastin. On the other hand, the high rate of the elasto-calcinosis of the aorta can be correlated with a significant increase in dicarboxylic amino-acids.

It is tempting to speculate that the increased calcium binding capacity of the elastin from the older specimens is somehow related to the increased content of free carboxyl groups. Two mechanisms by which these changes could be related suggest themselves. It is possible that the calcium ion is bound by the free carboxyl groups of aspartic and glutamic acids and is then precipitated as calcium phosphate or some other phosphate-containing calcium complex because of the smaller dissociation constants of the inorganic complexes. The carboxyl group could then again become available for the binding of more calcium. Another possibility is that the calcium is bound by the free carboxyl groups, leaving one valence available for combining with phosphate, which could then bind more calcium, etc. No direct stoichiometric relationship exists between the free carboxyl groups and the calcium, the latter being found in great molar excess per unit weight of the old aortic elastin.

Summary. Comparative analyses of elastin from young and old aortas reveal a pattern of characteristic age changes consisting of increase in specific gravity, calcium, and free carboxyl groups. There is an underlying shift in relative amino acid composition. In contrast to the aorta, the pulmonary artery shows minimal age changes.

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Fixed Rabies Virus in Blood Following Intracerebral Inoculation in Mice and Rabbits. (18605)

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There is little information on the distribution of viruses injected into the brain of animals. Schaeffer and Muckenfuss(1) studied the distribution of particulate matter introduced in the brain of monkeys by injecting India ink. They found only a very small part of the injected material at the site of its deposition. Schlesinger(2) observed that only 3 to 10% of the estimated amount of virus was recoverable from the infected brains of mice one hour after intracerebral inoculation of the Western equine encephalitis virus or

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