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Research Article

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The Interaction of Serum and Arterial Lipoproteins with Elastin of the Arterial Intima and Its Role in the Lipid Accumulation in Atherosclerotic Plaques

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ABSTRACT Arterial elastin appears to be a protein-lipid complex with the lipid component being bound to elastin peptide groups. In atherosclerotic lesions the lipid content of elastin increases progressively with increasing severity of atherosclerosis. The increases in the lipid content of plaque elastin are mainly due to large increases in cholesterol with about 80% of the cholesterol being cholesterol ester. This deposition of cholesterol in elastin accounts for a substantial part of the total cholesterol accumulation in atherosclerotic lesions of all stages. The present in vitro study suggests that the mechanism involved in the deposition of lipids in arterial elastin may be an interaction of the elastin protein with serum or arterial low density or very low density lipoproteins (LDL and VLDL) resulting in a transfer of lipids, but not of lipoprotein protein to the elastin. No significant lipid transfer occurred from the high density lipoproteins or chylomicrons. The amount of lipid taken up by plaque elastin was strikingly higher than by normal elastin and consisted mainly of cholesterol with over 80% of the cholesterol being cholesterol ester. The precondition for the lipid accumulation in plaque elastin appeared to be an altered amino acid composition of the elastin protein consisting of an increase in polar amino acids and a reduction in cross-linking amino acids. Subsequent treatment of lipoprotein-incubated arterial elastin with hot alkali and apolipoproteins did not reverse the binding of lipoprotein lipid to diseased elastin.

INTRODUCTION

Arterial elastic membranes appear to play an important role in the development of atherosclerotic plaques (1–9). In previous autoradiographic studies after intravenous injection of [^{3}H] cholesterol (10), dense accumulations of radioactive cholesterol occurred on the split and fragmented intimo-medial elastic membranes but not over collagen depositions in atherosclerotic plaques of all stages. These plaques included very small microscopic lesions which did not contain lipid in the cells of the slightly raised intima by autoradiography or lipid staining.

Recent biochemical analyses of arterial elastin preparations have shown that the lipids accumulating on elastic membranes of atherosclerotic lesions are associated with the elastin protein of these elastic membranes, even in early atherosclerosis (11). The arterial elastin preparations were defined as that protein material which remained undegraded after alkali hydrolysis of arterial tissue homogenates. The studies suggest that arterial elastin is a protein-lipid complex with the lipids being bound to the elastin peptides. The composition of this protein-lipid complex is altered in elastin of atherosclerotic plaques. As compared with normal elastin, plaque elastin has a significantly higher content of polar amino acids and a significantly lower content of cross-linking amino acids. The alteration of plaque elastin protein is associated with a higher content of lipids, mainly ester cholesterol, which increases progressively with increasing severity of atherosclerosis. The accumulation of cholesterol in plaque elastin accounts for about 30% of the total intimal cholesterol in well-developed plaques. In contrast, the abundant structural collagen present in atherosclerotic lesions appears to contribute only on a minor scale to the lipid accumulation in plaques. When homogenates of plaque intimae were treated with collagenase less than 8% of the total intimal cholesterol was released from the plaque tissue by the digestion of the plaque collagen.[1]

In mild and moderate atherosclerosis the elastin changes are limited to the plaque areas of the arterial intimae while the composition of elastin in the adjoining intimae and mediae remains unaltered. In severe athero-

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[1] Hollander, W., and D. M. Kramsch. Unpublished data.

sclerosis the lipid accumulation appears to spread into the intimae adjacent to advanced plaques as well as into the mediae below these plaques involving primarily the elastin of the elastic membranes in these areas. The present study was undertaken to explore the mechanism involved in the lipid deposition in arterial elastin, especially in the altered elastin of atherosclerotic lesions.

METHODS

Elastin Preparation

Five grossly normal and five atherosclerotic human aortas were obtained at autopsy within 4 h after death and immediately prepared for histological and biochemical studies. Macroscopically the atherosclerotic lesions in two aortas consisted of fatty streaks and dots (grade I plaques), whereas in the three other atherosclerotic aortas larger confluent lesions prevailed (grade II plaques). None of the plaques were complicated by ulceration, thrombosis, or gross calcification. Before preparation of the aortas for biochemical studies, small segments of normal aortas, plaques, and adjacent normal-appearing aortic areas were cut out and processed for histology as previously described (12).

The aortas were then stripped of their adventitias and medical layers. The intimal layers of atherosclerotic aortas were separated into plaque areas and into adjacent grossly normal areas which on microscopic examination also appeared to be normal. The plaque areas and adjacent normal intimae were pooled separately for each aorta and then minced and homogenized. Elastin was prepared from each set of tissue homogenates by boiling with 0.1 N NaOH according to a modification of the method of Lansing, Rosenthal, Alex, and Dempsey (13) omitting the methanol and acetone extractions before the base treatment. Similarly, the elastin was prepared from the intimal layers of the five normal aortas. The elastin preparations then were delipidated with chloroform-methanol 2:1 (vol/vol) and analyzed for their protein and lipid content as well as their amino acid and lipid composition. Elastin of similar amino acid composition was obtained when the tissue homogenates were delipidated before boiling in hot alkali. The procedures employed for elastin extraction and delipidation as well as for analysis of elastin protein, amino acids, and lipids are described in detail elsewhere (11).

Lipoprotein Preparation

Serum lipoproteins. Blood samples were obtained from normal and hyperlipoproteinemic human subjects after a 14-h overnight fast. The hyperlipemic blood was drawn from patients with types II, IV, and V hyperlipoproteinemia, as defined by Fredrickson, Levy, and Lees (14–16). The blood was collected in 50-ml Lusteroid tubes and allowed to clot at 26°C for approximately 1 h. Serum was separated by centrifugation at 2400 rpm for 30 min at 4°C. 1 ml of 1% disodium ethylenediaminetetraacetic acid (EDTA) was added to each 100 ml of serum and the serum then was processed immediately. The normal and hyperlipoproteinemic sera were identified by lipid analysis and typing by paper electrophoresis from 2-ml portions of each serum.

From the remaining portions of the sera the lipoproteins were separated by differential density ultracentrifugation according to the method of Havel, Eder, and Bragdon (17). The density of the samples was raised by addition of NaCl and KBr and checked by pycnometry at 20°C. For separation of chylomicrons the serum was left standing overnight and then spun at 9800 *g* for 10 min at 4°C. The other lipoprotein fractions were isolated by centrifuging the serum at 4°C in a Spinco Model L2 preparatory ultracentrifuge (Spinco Div., Beckman Instruments, Inc., Palo Alto, Calif.) using a 40-rotor head at 105,000 *g* for 22 h. The Lusteroid tubes were sliced with a standard tube slicer through the middle of the clear zone between the lipoprotein fractions contained in the supernatant and infranatant. The serum very low density lipoproteins (VLDL),[2] low density lipoproteins (LDL), and high density lipoproteins (HDL) were isolated at increasing solvent densities (*d*) of 1.006, 1.063, and 1.210 respectively. The isolated lipoprotein fractions were centrifuged twice more in salt solutions at each separation density to remove contaminants. All fractions were dialyzed at 4°C against 0.15 M NaCl containing 0.001 M EDTA at pH 7. They were characterized chemically as well as by paper electrophoresis and by immunoelectrophoresis.

Arterial lipoproteins. Human aortas showing mild to severe atherosclerosis were removed at autopsy within 4 h after death. The tissues were maintained at 4°C and processed immediately. The aortas were washed extensively in 0.85% saline to remove surface contamination by serum. The intimal layer then was peeled from the media of the aorta and again washed in normal saline. The lipoproteins were extracted from the intima according to methods previously described (18). The intimal samples weighing between 5 and 10 g (wet wt) were cut into small pieces of about 2×2 mm and homogenized for 15 s in 1.65 M NaCl containing 0.05 EDTA at pH 7.2. After the initial homogenization the homogenates were maintained at 4°C and extracted for 24 h at pH 7.2 with 1.65 M NaCl containing 0.05% EDTA using 10 ml of the NaCl solution per gram of tissue. The extracted homogenates then were centrifuged at 600 *g* for 10 min to remove tissue debris and the supernatant was saved. The sediment was resuspended in the same volume of the 1.65 M NaCl solution as before, extracted for another 24 h, and centrifuged in the same manner. The supernatants of the two extractions were then combined, dialyzed at 4°C against 0.15 M NaCl containing 0.05% EDTA at pH 7, and separated into lipoprotein fractions by the same procedures described for the serum lipoproteins.

Incubation Studies

The delipidated elastin from the intima of normal aorta, from plaques, and from adjacent normal intimae were then incubated with the lipoprotein fractions prepared from the normal and hyperlipoproteinemic sera and with the lipoprotein fractions extracted from the aortic intimae. Elastin samples containing 50 mg of delipidated elastin protein were incubated in a shaking incubator in Teflon-coated flasks at 37°C with 20 ml incubation medium containing 10 ml of the respective lipoprotein fraction in a phosphate buffer (0.0135 M KH_2PO_4, 0.0466 M Na_2HPO_4) at pH 7.35. The duration of the incubations ranged from 1 to 24 h. In some experiments the delipidated elastin was incubated with LDL and VLDL labeled with ^{125}I according to the method of Gitlin et al. (19). It was calculated that the iodinated lipoprotein contained about 0.8 atoms of iodine per molecule. The efficiency of iodination averaged 15%.

After the incubations the contents of the flasks were transferred to 30-ml glass tubes and spun at 3000 rpm in an International centrifuge (International Equipment Co.,

[2] *Abbreviations used in this paper:* HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins.

TABLE I

Protein and Lipid Composition of Elastin from the Intimal Layers of Normal and Atherosclerotic Aorta (mg/g elastin)

	Atherosclerotic aorta				
	Plaque intimae		Normal intimae adjacent to		
	Grade I plaques*	Grade II plaques‡	Grade I plaques*	Grade II plaques‡	Intimae of normal aortas§
Protein	869.2	777.2	988.7	985.2	986.9
Total lipid	130.8	222.8	11.3	14.8	13.1
Ester cholesterol	95.8	176.2	5.7	6.3	7.5
Free cholesterol	17.8	30.1	1.0	1.7	1.3
Phospholipids	8.4	10.5	1.2	2.2	1.3
Triglycerides	8.8	6.0	3.4	4.6	3.0

* Average value of two aortas with grade I plaques.

‡ Average values of three aortas with grade II plaques.

§ Average values of five normal aortas.

Needham Heights, Mass.) for 20 min at 25°C. The resultant supernatants were collected by pipetting and the elastin residues were washed two times with buffer for 10 min, three times with normal saline for 10 min and three times with distilled water for 10 min with centrifugation of the elastin after each washing step. The elastin was then dehydrated and delipidated as previously described (11) and analyzed for lipid and protein content and composition. In some experiments, after incubation with LDL and VLDL, the elastin was incubated with trypsin[3] for 18 h or treated a second time with boiling 0.1 N NaOH for 30 min.

The incubation experiments were controlled as follows. Lipoprotein fractions of normal and hyperlipoproteinemic serum were incubated in the same manner for 1, 4, and 24 h with 50 mg of sand instead of elastin. After incubation and washing no lipid was found to be associated with the sand after any of the incubation periods. Also, no protein was found in the sand after 1 or 4 h of incubation. However, after 24 h traces of the lipoprotein protein (1.4%) had sedimented with the sand.

The possibility that prolonged incubation might alter the lipoproteins facilitating the release of cholesterol also was tested in control experiments. Serum LDL and VLDL was preincubated for 24 h as described above with and without sand and with no elastin present in the incubation flasks. Analysis of the lipoproteins before and after incubation showed no change in their lipid and protein content except that negligible amounts of lipoprotein protein became insoluble as described above in the incubation experiment with sand after 24 hr. The preincubated lipoproteins then were incubated with 50 mg delipidated normal and plaque elastin protein for 1, 4, and 24 h. The rate of transfer of lipids to elastin from the preincubated lipoproteins was comparable to that found with freshly prepared lipoproteins.

In separate experiments the capacity of serum HDL and delipidated serum lipoproteins to remove transferred lipids from elastin was tested. Delipidated normal and plaque elastin was incubated as described above with the serum LDL. After the incubation, the elastin which contained the transferred lipids was thoroughly washed and then was incubated for 24 h in a phosphate buffer of pH 7.35 at 37°C with intact serum HDL as well as delipidated HDL, LDL, and VLDL. The serum HDL was delipidated with ethanol-diethyl ether by the method of Scanu (20) and the serum LDL and VLDL were partially delipidated by the diethyl ether technique of Avigan (21).

The lipid and protein content of elastin and lipoproteins was analyzed before and after each incubation. The lipids were extracted with chloroform-methanol. Free and ester cholesterol were determined by the method of Schoenheimer and Sperry (22), triglycerides by the method of Van Handel and Zilversmit (23), and phospholipids by the method of Youngburg and Youngburg (24). The recovery of the incubated lipoprotein lipids from the elastin and the post-incubation media was about 98%.

The protein content of elastin was measured from defatted and dehydrated samples by the Kjeldahl method. Lipoprotein protein was determined by the method of Lowry, Rosebrough, Farr, and Randall (25). The recovery of the incubated lipoprotein protein from the elastin and post-incubation media was about 97%. The ^{125}I radioactivity of the incubated lipoprotein and elastin was assayed before and after incubation in a deep well-type sodium iodide crystal scintillation counter by methods previously described (26). Before and after incubation samples of the elastin protein were hydrolyzed in a vacuum oven with 6 N HCl at 110°C for 24 h. This method of hydrolysis destroys any tryptophan present in the samples. After hydrolysis of the elastin protein samples amino acid composition was determined with a Technicon amino acid auto-analyzer (Technicon Instruments Corp., Tarrytown, N. Y.) according to the method of Hamilton (27).

RESULTS

Histology of Aortas

The grossly normal aortas showed a normal structure on microscopic examination. The grossly normal aortic areas adjacent to plaques also were microscopically normal. Grade I plaques showed a thickened intima con-

[3] Obtained from Worthington Biochemical Corp., Freehold, N. J.

TABLE II

Amino Acid Composition of Intimal Elastin from Normal and Atherosclerotic Aortas Used in Incubation Experiments (residues/1000 residues)

	Atherosclerotic aorta				
	Plaque intimae		Normal intimae adjacent to		
	Grade I plaques*	Grade II plaques‡	Grade I plaques*	Grade II plaques‡	Intimae of normal aortas§
Cysteic acid	0.5	1.4	1.3	1.5	1.1
Hydroxyproline	13.6	13.7	14.1	14.6	12.4
Aspartic acid	16.6	22.0	7.2	6.1	5.1
Threonine	16.6	16.8	10.8	12.3	11.3
Serine	17.2	16.4	9.2	10.1	9.6
Glutamic acid	34.3	40.9	23.1	22.1	21.8
Proline	132.2	121.3	128.3	132.8	129.6
Glycine	260.9	263.3	267.0	267.5	288.4
Alanine	200.7	198.6	220.1	226.1	215.9
Valine	127.7	125.2	131.5	132.3	131.3
Isoleucine	24.6	26.4	29.3	27.3	27.2
Leucine	62.9	63.5	67.8	65.0	64.7
Tyrosine	25.5	23.5	27.2	26.2	23.7
Phenylalanine	28.1	25.9	29.1	26.1	26.3
¼ Isodesmosine	3.2	3.1	5.8	4.5	5.1
¼ Desmosine	3.4	3.4	7.4	7.0	7.3
½ Lysininorleucine	0.3	0.1	1.3	0.9	1.2
Lysine	13.0	14.2	8.8	7.2	7.6
Histidine	4.8	5.1	2.6	1.9	2.4
Arginine	14.2	15.2	8.6	7.8	8.1

* Average values of two aortas with grade I plaques.
‡ Average values of three aortas with grade II plaques.
§ Average values of five normal aortas.

sisting mainly of lipid-filled cells with very little deposition of collagen fibers. The intimal elastic membranes of these plaques frequently revealed splitting and fragmentation at several points and often showed depositions of stainable lipids. In grade II plaques the intimal elastic membranes showed severe destruction and deposition of stainable lipids. The areas of intimal thickening in these plaques were larger and consisted of lipid-filled cells, many of them ruptured, and of extensive collagen depositions.

Chemical Composition of the Intimal Elastin Preparation

Protein and lipid composition. Table I shows the protein and lipid composition of normal and plaque elastin which was used, after delipidation, for the lipoprotein incubation experiments. Plaque elastin of grade I plaques had a lipid content which was about eight times higher than that of elastin from normal aortic intimae, whereas the lipid content of plaque elastin from grade II plaques was about 16–18 times higher. The increases in lipid content of plaque elastin were mainly due to large increases in cholesterol with about 80% of the cholesterol being cholesterol ester. The increases in phospholipids and triglycerides were comparatively smaller. The intensive staining of isolated plaque elastin with Oil Red O also was consistent with its high lipid content.

Elastin from normal intimal areas adjacent to plaques had a protein and lipid content which was comparable to that of elastin from the intima of normal aorta. The lipid content of normal intimal elastin was small and accounted for the weak staining of isolated normal elastin with Oil Red O. The major lipid component of normal and adjacent normal elastin also was cholesterol with over 75% of the cholesterol being cholesterol ester.

Amino acid composition. Table II shows the amino acid composition of normal and plaque elastin. As compared with elastin of normal intima, plaque elastin showed marked increases in the following polar amino acids: aspartic acid, threonine, serine, glutamic acid, lysine, histidine, and arginine. The cross-linking amino acids isodesmosine, desmosine, and lysinonorleucine were strikingly reduced in plaque elastin. The elastin from normal-appearing intimal areas adjacent to plaques was

TABLE III

Lipid Uptake by Delipidated Normal and Plaque Elastin of Aortic Intimae Incubated with Lipoprotein Fractions of Normal Human Sera

	Lipoprotein fractions of serum A				Lipoprotein fractions of serum B			Lipoprotein fractions of serum C		
	Medium* before incubation	Elastin after incubation‡			Medium* before incubation	Elastin after incubation‡		Medium* before incubation	Elastin after incubation‡	
		Grade I plaque	Adjacent normal	Normal§		Grade II plaque	Adjacent normal		Grade II plaque	Adjacent normal
d < 1.006 (VLDL)										
Ester cholesterol	3.5‖	2.6	0.9	0.8	4.1	3.1	1.1	5.4	4.1	1.3
Free cholesterol	1.3	0.3	0.1	0.1	1.5	0.3	0.1	1.8	0.4	0.1
Phospholipids	3.9	1.2	0.2	0.3	3.8	1.1	0.2	3.6	1.1	0.2
Triglycerides	19.6	2.6	1.7	1.0	22.2	2.7	1.8	30.6	2.3	1.7
d 1.006–1.063 (LDL)										
Ester cholesterol	10.8	8.1	2.0	1.8	12.4	9.5	2.4	8.7	6.1	1.7
Free cholesterol	3.1	0.5	0.1	0.1	2.5	0.4	0.1	4.9	0.7	0.2
Phospholipids	6.6	2.0	1.2	0.9	7.3	2.2	1.3	5.5	2.1	1.0
Triglycerides	3.5	1.4	0.6	0.7	3.2	1.3	0.6	3.6	1.6	0.7
d 1.063–1.210 (HDL)										
Ester cholesterol	3.8	0.0	0.0	0.0	3.5	0.0	0.0	2.4	0.0	0.0
Free cholesterol	0.4	0.0	0.0	0.0	0.4	0.0	0.0	0.5	0.0	0.0
Phospholipids	7.0	0.4	0.2	0.2	7.3	0.4	0.3	8.5	0.5	0.3
Triglycerides	1.8	0.0	0.0	0.0	1.3	0.0	0.0	2.4	0.0	0.0

* Represents total lipid content in milligrams per 20 ml volume of incubation medium for each lipoprotein fraction.
‡ Represents total lipid uptake in milligrams by 50 mg elastin protein from each incubation medium.
§ Elastin from intima of normal aorta.
‖ The figures represent single experiments.

normal as indicated by its amino acid composition which was comparable to that of intimal elastin from normal aortas.

INCUBATION OF DELIPIDATED INTIMAL ELASTIN WITH LIPOPROTEINS

Incubation with lipoproteins of normal human serum. Table III compares the lipid uptake of delipidated normal and plaque elastin after incubation with lipoprotein fractions of normal serum for 24 h. After incubation with lipoproteins of *d* < 1.006 (VLDL) or *d* 1.006–1.063 (LDL), about 71–76% of the lipoprotein ester cholesterol was transferred to plaque elastin. The transfer of ester cholesterol to normal elastin was much smaller and amounted to about 25% from the VLDL and about 15% from the LDL fraction. Smaller amounts of free cholesterol, phospholipids, and triglycerides were transferred to both normal and plaque elastin from the VLDL and LDL fractions with the uptake by plaque elastin being higher than by normal elastin. The difference in lipid transfer to normal and plaque elastin after incubation with LDL and VLDL also was demonstrable by staining of the incubated elastin with Oil Red O and Sudan IV. In contrast to the lipid transfer from the VLDL and LDL fractions, there was no uptake of lipids by normal or plaque elastin from the lipoprotein fraction *d* 1.063–1.210 (HDL) with the exception of small amounts of phospholipids.

Incubation with serum lipoproteins of patients with type II hyperlipoproteinemia. Table IV shows the lipid uptake by delipidated normal and plaque elastin after incubation for 24 h with serum lipoprotein fractions of patients with type II hyperlipoproteinemia. After incubation with the VLDL or LDL fractions of type II sera about 71–75% of the lipoprotein ester cholesterol was transferred to plaque elastin, whereas the transfer to normal elastin was much smaller. Although the percentage of the ester cholesterol transfer from the LDL fractions of type II sera to plaque elastin was about the same as from the LDL fractions of normal serum, the absolute amount of cholesterol ester taken up by plaque elastin was strikingly higher than from the LDL of normal serum. The uptake of free cholesterol, phospholipids, and triglycerides from the VLDL and LDL fractions by normal and plaque elastin was comparatively small. However, the transfer of these lipids from the LDL fractions of type II sera to plaque elastin also was somewhat higher than from the LDL fractions of normal serum. Since the serum LDL fractions of type II sera had a higher concentration of ester and free cholesterol, phospholipids, and triglycerides than the LDL fractions of normal serum it appears that the transfer of lipids to plaque elastin is influenced by the LDL concentration. In contrast, the transfer of lipids to normal elastin did not appear to be dependent on the LDL concentration in the incubation medium. As with the

TABLE IV

Lipid Uptake by Delipidated Normal and Plaque Elastin of Aortic Intimae Incubated with Serum Lipoprotein Fractions of Patients with Hyperlipoproteinemia Type II

	Lipoprotein fractions of serum D				Lipoprotein fractions of serum E			Lipoprotein fractions of serum F		
	Medium* before incubation	Elastin after incubation‡			Medium* before incubation	Elastin after incubation‡		Medium* before incubation	Elastin after incubation‡	
		Grade I plaque	Adjacent normal	Normal§		Grade II plaque	Adjacent normal		Grade II plaque	Adjacent normal
a < 1.006 (VLDL)										
Ester cholesterol	4.3‖	3.2	0.9	1.2	4.8	3.5	1.0	5.7	4.3	1.1
Free cholesterol	1.5	0.3	0.1	0.1	2.7	0.6	0.2	2.0	0.4	0.1
Phospholipids	4.8	1.4	0.2	0.1	5.3	1.6	0.3	4.9	1.5	0.3
Triglycerides	29.3	1.9	1.0	1.3	34.9	2.9	1.5	40.1	2.2	1.3
d 1.006–1.063 (LDL)										
Ester cholesterol	17.6	13.0	1.8	2.0	20.3	14.4	2.0	23.0	16.6	2.3
Free cholesterol	7.2	1.8	0.2	0.2	8.4	2.1	0.3	9.7	2.4	0.3
Phospholipids	13.5	3.4	1.3	1.2	16.6	4.1	1.4	17.8	4.2	1.3
Triglycerides	6.7	2.1	0.9	1.5	8.4	2.5	1.0	13.2	4.2	1.7
d 1.063–1.210 (HDL)										
Ester cholesterol	2.4	0.0	0.0	0.0	4.8	0.0	0.0	4.9	0.0	0.0
Free cholesterol	0.5	0.0	0.0	0.0	0.9	0.0	0.0	0.7	0.0	0.0
Phospholipids	11.6	0.4	0.1	0.2	10.2	0.3	0.1	12.1	0.4	0.2
Triglycerides	1.8	0.0	0.0	0.0	0.9	0.0	0.0	1.5	0.0	0.0

* Represents total lipid content in milligrams per 20 ml volume of incubation medium for each lipoprotein fraction.
‡ Represents total lipid uptake in milligrams by 50 mg elastin protein from each incubation medium.
§ Elastin from intima of normal aorta.
‖ The figures represent single experiments.

TABLE V

Lipid Uptake by Delipidated Normal and Plaque Elastin of Aortic Intimae Incubated with Serum Lipoprotein Fractions of Patients with Hyperlipoproteinemia Type IV

	Lipoprotein fractions of serum G				Lipoprotein fractions of serum H		
	Medium* before incubation	Elastin after incubation‡			Medium* before incubation	Elastin after incubation‡	
		Grade I plaque	Adjacent normal	Normal§		Grade II plaque	Adjacent normal
d < 1.006							
Ester cholesterol	22.5‖	17.1	4.5	4.2	39.9	29.8	2.3
Free cholesterol	12.4..	2.6	0.5	0.3	22.0	4.4	0.3
Phospholipids	9.4	2.8	0.4	0.5	13.1	3.9	0.4
Triglycerides	187.4	7.3	2.1	2.3	294.4	8.8	1.9
d 1.006–1.063							
Ester cholesterol	7.6	5.5	0.8	0.6	6.2	4.5	0.5
Free cholesterol	2.3	0.6	0.2	0.4	2.6	0.7	0.1
Phospholipids	4.5	1.7	0.3	0.2	4.7	1.5	0.4
Triglycerides	6.8	2.0	0.7	0.8	5.1	1.6	0.6
d 1.063–1.210							
Ester cholesterol	5.3	0.0	0.0	0.0	1.7	0.0	0.0
Free cholesterol	0.8	0.0	0.0	0.0	0.3	0.0	0.0
Phospholipids	3.8	0.4	0.2	0.1	6.9	0.3	0.1
Triglycerides	2.5	0.0	0.0	0.0	1.5	0.0	0.0

* Represents total lipid content in milligrams per 20 ml volume of incubation medium for each lipoprotein fraction.
‡ Represents total lipid uptake in milligrams by 50 mg elastin protein from each incubation medium.
§ Elastin from intima of normal aorta.
‖ The figures represent single experiments.

TABLE VI

Lipid Uptake by Delipidated Normal and Plaque Elastin of Aortic Intimae Incubated with Serum Chylomicron Fractions of Patients with Hyperlipoproteinemia Type V

	Chylomicron fraction A			Chylomicron fraction B		
	Medium* before incubation	Elastin after incubation‡		Medium* before incubation	Elastin after incubation‡	
		Grade I plaque	Adjacent normal		Grade II plaque	Adjacent normal
Ester cholesterol	4.0§	0.2	0.1	2.6	0.1	0.0
Free cholesterol	5.3	0.1	0.0	3.4	0.0	0.0
Phospholipids	9.3	0.5	0.1	6.0	0.3	0.1
Triglycerides	111.3	1.1	1.0	71.9	0.8	0.8

* Represents total lipid content in milligrams per 20 ml incubation medium.

‡ Represents total lipid uptake in milligrams by 50 mg elastin protein from incubation media.

§ The figures represent single experiments.

HDL fraction of normal serum, there was no lipid transfer from the HDL fraction of type II sera to normal or plaque elastin except for small transfers of phospholipids.

Incubation with serum lipoproteins of patients with type IV hyperlipoproteinemia. Table V shows the lipid uptake by delipidated normal and plaque elastin after incubation for 24 h with serum lipoprotein fractions of patients with type IV hyperlipoproteinemia. The percentages of lipid transfers from the VLDL and LDL fraction of type IV sera to normal and plaque elastin was comparable to those from the same fractions of normal serum with the exception of triglycerides from the VLDL fraction. The percentage of triglycerides taken up by plaque elastin from the VLDL fraction was smaller than from the VLDL fraction of normal serum. However, the absolute amount of triglyceride transferred to plaque elastin from the VLDL fraction of type IV sera was greater than from the same fraction of normal serum. Likewise, the absolute amounts of ester and free cholesterol and phospholipids transferred from the VLDL fraction to plaque elastin were higher than from the VLDL fraction of normal serum. The large transfer of lipids to plaque elastin appeared to be due to their higher concentration in the VLDL fraction of type IV sera. From the HDL fraction of type IV sera no lipid was taken up by normal or plaque elastin except for small amounts of phospholipid.

Incubation with the serum chylomicron fraction of patients with type V hyperlipoproteinemia. As shown in Table VI there was very little or no lipid uptake by delipidated normal or plaque elastin after incubation for 24 h with the serum chylomicron fraction. It is noteworthy that the transfer of chylomicron triglycerides in contrast to VLDL and LDL triglycerides was small despite its high concentration in the chylomicron fractions.

Incubation with arterial lipoproteins. Table VII shows the lipid uptake by delipidated normal and plaque elastin after incubation for 24 h with the lipoprotein fractions isolated from the aortic intimae. The transfers of lipids from the arterial lipoprotein fractions were comparable to those from serum lipoprotein fractions. The transfer of ester cholesterol from the arterial lipoprotein fractions $d < 1.006$ and d $1.006 - 1.063$ to plaque elastin ranged between 70 and 77% of the ester cholesterol in these fractions whereas the transfer of ester cholesterol to normal elastin was about 15–27%. Smaller amounts of free cholesterol, phospholipids, and triglycerides were taken up by both normal and plaque elastin with the transfer of these lipids to plaque elastin being somewhat higher than to normal elastin. As with the HDL fractions of the sera, no lipid was taken up by normal or plaque elastin from the arterial HDL fractions except for small amounts of phospholipids.

Time-course of ester cholesterol transfer from serum LDL and VLDL to intimal elastin. Fig. 1 shows the uptake of ester cholesterol by delipidated normal and plaque elastin after 1 h, 4, and 24 h of incubation with the LDL fractions of normal serum and of serum from patients with type II hyperlipoproteinemia. The uptake of ester cholesterol by plaque elastin increased progressively with time with about 75% of the cholesterol ester being transferred after 24 h from both LDL fractions. However, the absolute amounts of cholesterol ester transferred to plaque elastin at all incubation times were greater with LDL of the type II serum than with LDL of normal serum. The uptake of ester cholesterol by normal elastin from both LDL fractions approached a maximum after 4 h of incubation with little or no additional transfer thereafter. The amounts of ester cholesterol taken up by normal elastin from the LDL of normal or hypercholesterolemic serum were small and of about equal magnitude.

TABLE VII

Lipid Uptake by Normal and Plaque Elastin of Aortic Intimae Incubated with Tissue Lipoprotein Fractions Extracted from Human Aortas

	Lipoprotein fractions of extract A				Lipoprotein fractions of extract B		
		Elastin after incubation‡				Elastin after incubation‡	
	Medium* before incubation	Grade I plaque	Adjacent normal	Normal§	Medium* before incubation	Grade II plaque	Adjacent normal
d < 1.006							
Ester cholesterol	6.6‖	5.1	1.8	1.6	7.3	5.6	1.4
Free cholesterol	1.2	0.3	0.1	0.1	1.8	0.4	0.1
Phospholipids	1.7	0.3	0.2	0.2	2.1	0.3	0.2
Triglycerides	2.9	0.7	0.2	0.1	3.6	1.1	0.4
d 1.006–1.063							
Ester cholesterol	4.5	3.4	1.0	1.1	10.6	7.5	1.6
Free cholesterol	3.6	0.7	0.1	0.1	7.5	1.3	0.3
Phospholipids	3.3	1.0	0.3	0.2	4.8	1.3	0.6
Triglycerides	1.0	0.3	0.1	0.0	2.3	0.9	0.2
d 1.063–1.210							
Ester cholesterol	3.4	0.0	0.0	0.0	3.6	0.0	0.0
Free cholesterol	0.1	0.0	0.0	0.0	0.1	0.0	0.0
Phospholipids	10.6	0.3	0.1	0.1	10.7	0.4	0.1
Triglycerides	0.0	0.0	0.0	0.0	0.2	0.0	0.0

* Represents total lipid content in milligrams per 20 ml volume of incubation medium for each lipoprotein fraction.

‡ Represents total lipid uptake in milligrams by 50 mg elastin protein from each incubation medium.

§ Elastin from intima of normal aorta.

‖ The figures represent single experiments.

It is of interest that no lipoprotein protein was found to be associated with normal or plaque elastin after 4 h of incubation with both LDL fractions. At that time plaque elastin had already taken up about 30% and normal elastin about 90% of the total uptake of ester cholesterol. However, after continued incubation to 24 h small amounts of lipoprotein protein from both LDL fractions appeared to be associated with normal and plaque elastin. Comparable results were obtained when normal and plaque elastin was incubated for 1, 4, and 24 h with VLDL fractions of normal serum and of serum from patients with type IV hyperlipoproteinemia.

Effect of trypsin on lipoprotein-incubated elastin. Table VIII shows the uptake of ester cholesterol and protein by delipidated normal and plaque elastin after incubation for 24 h with serum LDL labeled with ^{125}I and subsequent treatment with trypsin. After the lipoprotein incubation about 75% of the LDL cholesterol ester was transferred to plaque elastin whereas about 24% of the LDL protein, labeled and nonlabeled, was associated with the plaque elastin preparation. Normal elastin took up about 20% of the LDL ester cholesterol and about 17% of the LDL protein. However, the actual amounts of LDL protein associated with the intimal elastin were so small that no changes in the amino acid composition of normal or plaque elastin could be detected after incubation.

After subsequent incubation with trypsin all of the LDL protein was removed but virtually all of the transferred cholesterol ester remained associated with the elastin. Similar findings were obtained after incubation

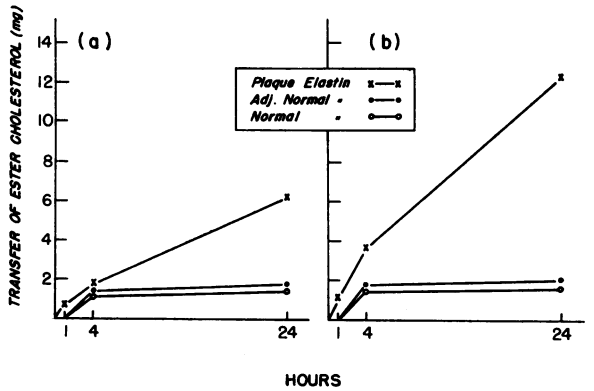

FIGURE 1 Time-course of ester cholesterol transfer from serum low density lipoproteins (*d* 1.006–1.063) to normal and plaque elastin. (Averages of three experiments.) (*a*) Ester cholesterol transfers from LDL of Normal human serum. (*b*) Ester cholesterol transfers from LDL of serum of a patient with hyperlipoproteinemia type II.

TABLE VIII

Interaction of Delipidated Normal and Plaque Elastin with Serum Low Density Lipoprotein (1.006–1.063) after Incubation for 24 and the Effect of Trypsin on the Incubated Elastin

		Grade II plaque elastin‡		Adjacent normal elastin‡	
	Medium* before incubation	Uptake after incubation	Remaining after trypsin	Uptake after incubation	Remaining after trypsin
Lipoprotein ester cholesterol (*mg*)	8.7§	75.3%	73.8%	20.6%	19.1%
Lipoprotein protein (*mg*)	4.3	24.6%	0.0%	17.2%	0.0%
^{125}I-labeled lipoprotein (*cpm* × 10^6)	9.7	24.1%	0.5%	16.5%	0.3%

* Volume of incubation medium = 20 ml.
‡ 50 mg of elastin protein.
§ Averages of three experiments.

of normal and plaque elastin with serum VLDL and subsequent treatment of the elastin with trypsin.

Effect of base treatment on lipoprotein-incubated elastin. Table IX shows the effect of alkali hydrolysis on normal and plaque elastin which previously had been incubated for 24 h with serum LDL labeled with ^{125}I. After the lipoprotein incubation the percent transfers of LDL ester cholesterol as well as of labeled and nonlabeled LDL protein to normal and plaque elastin were comparable to those of the LDL incubation in the trypsin experiment. After subsequent base treatment of the incubated elastin all of the LDL protein was removed from the elastin. However, about 32% of the LDL ester cholesterol remained associated with plaque elastin although about 20% of the elastin protein itself was hydrolyzed by the prolonged alkali treatment. It is noteworthy that after the base treatment all of the LDL ester cholesterol bound by elastin remained esterified.

Effect of apolipoproteins and of HDL on lipoprotein-incubated elastin. Table X shows the lipid content of normal and plaque elastin after incubation for 24 h with serum LDL and after subsequent incubation of the elastin with intact HDL as well as with apo-HDL, apo-LDL, and apo-VLDL. The lipids transferred to normal and plaque elastin after incubation with LDL were not removed by subsequent incubation of the elastin with intact HDL or with any of the delipidated lipoproteins.

DISCUSSION

The striking increase in lipid content, particularly of ester cholesterol, as well as the increase in polar amino acids and the reduction in cross-linking amino acids observed in the alkali-extracted elastin of grade I and grade II plaques, are in agreement with previous observations (11). The differences in the amino acid composition involve amino acids which represent relatively small components of the isolated elastin preparations. Increases in polar amino acids of elastin from atherosclerotic human aortas also have been reported by other workers (28).

The present in vitro study suggests that the mechanism involved in the deposition of lipids in arterial elastin may be an interaction of the elastin protein with low density and very low density lipoproteins. The pre-

TABLE IX

Interaction of Delipidated Normal and Plaque Elastin with Serum Low Density Lipoprotein (1.006–1.063) after Incubation for 24 h and the Effect of Base Treatment on the Incubated Elastin

		Grade II plaque elastin‡		Adjacent normal elastin‡	
	Medium* before incubation	Uptake after incubation	Remaining after alkali§	Uptake after incubation	Remaining after alkali§
Lipoprotein ester cholesterol (*mg*)	8.7‖	75.0%	31.8%	21.8%	10.1%
Lipoprotein protein (*mg*)	4.3	24.0%	0.0%	18.4%	0.0%
^{125}I-labeled lipoprotein (*cpm* × 10^6)	9.7	23.5%	0.4%	16.9%	0.1%

* Volume of incubation medium = 20 ml.
‡ 50 mg elastin protein.
§ Boiling 0.1 N NaOH for 30 min.
‖ Averages of three experiments.

TABLE X

Lipid Content of Normal and Plaque Elastin after Incubation with Serum Low Density Lipoprotein and after Subsequent Incubation with Intact HDL and Delipidated HDL, LDL, and VLDL of Human Serum

		Grade II plaque elastin‡					Adjacent normal elastin‡				
			Content after additional incubation with					Content after additional incubation with			
	Medium* of LDL incubation	Content after LDL incubation	Intact HDL	apo-HDL	apo-LDL	apo-VLDL	Content after LDL incubation	Intact HDL	apo-HDL	apo-LDL	apo-VLDL
Ester cholesterol (*mg*)	7.9§	5.9	5.9	5.8	5.9	5.9	1.9	1.8	1.9	1.9	1.9
Free cholesterol (*mg*)	3.5	0.8	0.7	0.6	0.8	0.8	0.4	0.4	0.3	0.4	0.4
Phospholipids (*mg*)	4.1	2.1	2.0	2.1	2.1	2.1	1.3	1.3	1.3	1.3	1.3
Triglycerides (*mg*)	2.7	0.8	0.5	0.8	0.8	0.8	0.5	0.5	0.3	0.5	0.5

* Represents total lipid content per 20 ml incubation medium.

‡ Represents 50 mg elastin protein for each incubation.

§ Averages of three experiments.

requisite for the capacity of plaque elastin to bind greater amounts of lipids than normal elastin appeared to be the alteration in the amino acid composition of elastin protein from plaques. The mechanism responsible for the compositional changes of plaque elastin protein are as yet not clear. As discussed in more detail elsewhere (11) two posible mechanisms may be considered. In brief, these two possible mechanisms are (*a*) formation of new but defective elastin replacing degenerated elastic tissue, and (*b*) firm binding of one or more secondary proteins to true elastin.

Recently reported studies (29) have provided further evidence that the shift in the amino acid composition of the alkali-insoluble protein from plaques is due to an alteration of the elastin itself and not to chance contamination by an extraneous protein which is equally resistant to alkali hydrolysis. In these studies an abnormal lipopeptide fraction was isolated from elastase digests of plaque elastin by column chromatography on Sephadex G200. It was not present in elastin from normal arterial intimae. This lipopeptide fraction, which was free of hexosamine, uronic acid, neuraminic acid, and calcium, was responsible for the binding of the large amounts of lipids to the elastin. As compared with normal elastin, the peptide moiety of this fraction contained markedly more aspartic acid, threonine, serine, glutamic acid, lysine, histidine, arginine, and somewhat more hydroxyproline, whereas proline, glycine, alanine, and valine were reduced; the content in desmosines, however, was about the same as in normal elastin indicating that the peptide was not derived from an extraneous protein. This abnormal lipopeptide fraction was not further separable by chromatography on Biogel 150 and migrated as a single band in polyacrylamide gel with the lipids migrating with the peptide. The presence of the typical elastin cross-links as well as of hydroxyproline also indicate that this lipopeptide was not identical with the fibrillar structural glycoprotein which according to Ross and Bornstein (30) is associated with elastin. However, it cannot be entirely excluded that this glycoprotein at least in part may play a role in the increase of polar amino acids in the abnormal lipopeptide of elastin from plaques. In that case one would have to postulate an unusually tight binding of this glycoprotein to plaque elastin to explain its resistance to alkali hydrolysis which normally destroys this protein. On the other hand, when binding of a secondary protein is considered as a possible mechanism for the shift in the amino acid composition of plaque elastin, this secondary protein could be any protein rich in polar amino acids. Proteins of this type would include other glycoproteins, lipoproteins, collagen, anti-elastin antibodies, and other immunoglobulins.

As reported by Mandl, Keller, and Levi (31) and Robert, Grosgogeat, Reverdy, Robert, and Robert (32) it appears that elastin can be immunogenic and is capable of eliciting anti-elastin antibodies. However, with regard to the other proteins mentioned two of these appear not to be very likely to contribute to the compositional changes of plaque elastin: collagen and the protein moiety of lipoproteins. As outlined previously (11) elastin was treated with collagenase after extraction with hot alkali. This procedure resulted in an elastin preparation with a hydroxyproline content which was too low to be contaminated by collagen. The present in vitro study suggests that the protein moiety of lipoproteins also may not be involved in the alterations of plaque elastin protein since the transfer of lipid to normal and plaque elastin appeared to occur independently from that of the protein moiety of the lipoprotein. After 4 h of incubation with low density lipoproteins there was no transfer of lipoprotein protein to the elastin. At that time plaque elastin had already taken up 30% and normal elastin 90%

of the total lipoprotein lipid which was contained in the elastin after 24 h of incubation. After 24 h of incubation small amounts of lipoprotein protein were found to sediment with the elastin. However, after subsequent treatment of the incubated elastin with trypsin, the lipoprotein protein was removed, whereas all of the transferred lipids were still associated with the elastin.

The lipid uptake by plaque elastin, especially of ester cholesterol, was strikingly higher than by normal elastin irrespective of whether the elastin was incubated with lipoproteins of normal serum or hyperlipoproteinemic serum. This finding may provide an explanation of why lipids, and especially ester cholesterol, accumulate in atherosclerotic lesions even when the lipid levels of the serum lipoproteins are normal. The present study also suggests that higher serum concentrations of lipids, as they occur in hyperlipoproteinemias with elevated VLDL and LDL levels, may result in accelerated lipid depositions in atherosclerotic intimae. In contrast, the lipid transfers to the normal intimal elastin of normal intimae did not appear to be dependent on the lipid concentrations of the lipoprotein fractions suggesting that elastin of normal protein composition does not incorporate more than its normal lipid content whether the circulating serum lipids are increased or not.

The uptake of cholesterol by human arterial elastin also has been demonstrated in vivo after intravenous injection of [^{3}H] cholesterol (11). The in vivo studies suggested that cholesterol from circulating lipoproteins may be transferred, mainly in the form of cholesterol ester, to normal and plaque elastin with the uptake by plaque elastin being about six times higher than by normal elastin. The present in vitro study also indicates that lipids are transferable from arterial lipoproteins as well as from serum lipoproteins to elastin.

The major lipid taken up by delipidated plaque elastin after incubation was cholesterol with over 80% of the transferred cholesterol being cholesterol ester. These findings are consistent with the lipid composition of alkali-extracted plaque elastin before incubation (and delipidation). Before incubation the major lipid component of the alkali-extracted plaque elastin (before delipidation) also was cholesterol with about 80% of the cholesterol being cholesterol ester.

The affinity of the lipoprotein lipids, especially of ester cholesterol, to arterial elastin appeared to be greater than their affinity to the protein moiety of the lipoproteins. After incubation with plaque elastin protein the lipoproteins had lost most of their ester cholesterol to the elastin and subsequent incubation with HDL, apo-HDL, apo-LDL, and apo-VLDL did not reverse the lipid transfer from normal or plaque elastin. Even subsequent treatment of lipoprotein-incubated elastin with hot alkali removed only a portion of the transferred lipids although part of the elastin protein itself was hydrolyzed by the alkali. The binding of the transferred ester cholesterol by plaque elastin also appeared to protect the esterified lipid from alkali hydrolysis since almost all of the LDL cholesterol still bound to the elastin after base treatment remained esterified. In contrast, when ultracentrifugally isolated LDL of serum or homogenates of fatty liver were subjected to treatment with hot alkali in the same manner, all the esterified lipids were completely hydrolyzed (11). Previous and recent studies after degradation of the elastin with elastase have shown that the elastin lipids are bound to the peptide moieties of the elastin (11, 29). These findings indicate that the binding of lipids to arterial elastin is firm and may not be reversible. Since in atherosclerotic lesions between 20 and 50% of the total intimal cholesterol may be bound to the altered elastin protein of the lesion (11), it seems questionable whether the lipid accumulating in the atherosclerotic intima can be removed entirely once the alteration of the elastin has been established.

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REFERENCES

1. Lansing, A. I., M. Alex, and T. B. Rosenthal. 1950. Atheromatosis as a sequel to senescent changes in the arterial wall. *J. Gerontal.* **5**: 314.
2. Moon, H. D., and J. F. Rinehart. 1952. Histogenesis of coronary arteriosclerosis. *Circulation.* **6**: 481.
3. Zugibe, F. T., and K. D. Brown. 1960. Histochemical studies in atherogenesis: human aortas. *Circ. Res.* **8**: 287.
4. Parker, F. 1960. An electron microscopic study of experimental atherosclerosis. *Am. J. Pathol.* **36**: 19.
5. Adams, C. W. M., and N. A. Tuqan. 1961. Elastic degeneration as source of lipids in the early lesions of atherosclerosis. *J. Pathol. Bacteriol.* **82**: 131.
6. Osborn, G. R. 1963. The Incubation Period of Coronary Thrombosis. Butterworth and Co., Ltd., London. 3–44.
7. Friedman, M. 1963. Pathogenesis of the spontaneous atherosclerotic plaque. A study on the cholesterol-fed rabbit. *Arch. Pathol.* **76**: 318.
8. Smith, E. B., P. H. Evans, and M. D. Downham. 1967. Lipid in the aortic intima. The correlation of morphological and chemical characteristics. *J. Atheroscler. Res.* **7**: 171.
9. Kramsch, D. M., and W. Hollander. 1968. Occlusive atherosclerotic disease of the coronary arteries in monkey (*Macaca irus*) induced by diet. *Exp. Mol. Pathol.* **9**: 1.
10. Kramsch, D. M., W. Hollander, and C. Franzblau. 1970. The role of arterial elastin in the lipid accumulation in human atherosclerotic arteries. *In* Atherosclerosis. Second International Symposium. R. J. Jones, editor. Springer-Verlag New York, Inc., New York. 115.
11. Kramsch, D. M., C. Franzblau, and W. Hollander. 1971. The protein and lipid composition of arterial elastin and its relationship to lipid accumulation in the atherosclerotic plaque. *J. Clin. Invest.* **50**: 1666.

12. Kramsch, D. M., I. Gore, and W. Hollander. 1967. The distribution of intravenously administered ^{3}H-cholesterol in the arteries and other tissues. Part 2. Radioautographic findings. *J. Atheroscler. Res.* **7**: 501.
13. Lansing, A. I., T. B. Rosenthal, M. Alex, and E. W. Dempsey. 1952. The structure and chemical characterization of elastic fibers as revealed by elastase and by electron microscopy. *Anat. Rec.* **114**: 555.
14. Fredrickson, D. S., R. I. Levy, and R. S. Lees. 1967. Fat transport in lipoproteins. An integrated approach to mechanisms and disorders. *N. Engl. J. Med.* **276**: 148.
15. Fredrickson, D. S., R. I. Levy, and R. S. Lees. 1967. Fat transport in lipoproteins. An integrated approach to mechanisms and disorders. *N. Engl. J. Med.* **276**: 215.
16. Fredrickson, D. S., R. I. Levy, and R. S. Lees. 1967. Fat transport in lipoproteins. An integrated approach to mechanisms and disorders. *N. Engl. J. Med.* **276**: 273.
17. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34**: 1345.
18. Hollander, W., D. M. Kramsch, and G. Inoue. 1968. The metabolism of cholesterol, lipoproteins, and acid mucopolysaccharides in normal and atherosclerotic vessels. *Prog. Biochem. Pharmacol.* **4**: 270.
19. Gitlin, D., D. G. Cornwell, D. Nakasato, J. L. Onclay, W. L. Hughes, Jr., and C. A. Janeway. 1958. Studies on the metabolism of plasma proteins in the nephrotic syndrome. II. The lipoproteins. *J. Clin. Invest.* **37**: 172.
20. Scanu, A. 1966. Forms of human serum high density lipoprotein protein. *J. Lipid Res.* **7**: 295.
21. Avigan, J. 1957. Modification of human serum lipoprotein fractions by lipid extraction. *J. Biol. Chem.* **226**: 957.
22. Schoenheimer, R., and W. M. Sperry. 1934. A micromethod for the determination of free and combined cholesterol. *J. Biol. Chem.* **106**: 745.
23. Van Handel, E., and D. B. Zilversmit. 1957. Micromethod for the direct determination of serum triglycerides. *J. Lab. Clin. Med.* **50**: 152.
24. Youngburg, G. E., and M. V. Youngburg. 1930. Phosphorus metabolism. I. A system of blood phosphorus analysis. *J. Lab. Clin. Med* **16**: 158.
25. Lowry, O. H., N. J. Rosebrough, A. I. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265.
26. Hollander, W., and D. M. Kramsch. 1967. The distribution of intravenously administered ^{3}H-cholesterol in the arteries and other tissues. Part 1. Tissue fractionation findings. *J. Atheroscler. Res.* **7**: 491.
27. Hamilton, P. B. 1963. Ion exchange chromatography of amino acids. A single column, high resolving, fully automatic procedure. *Anal. Chem.* **35**: 2055.
28. Yu, S. Y. 1971. Cross-linking of elastin in human atherosclerotic aortae. I. A preliminary study. *Lab. Invest.* **25**: 121.
29. Kramsch, D. M., and M. Gottwik. 1972. Isolation of an abnormal lipopeptide from elastin of the human atherosclerotic plaque. *Fed. Proc.* **31**: 219.
30. Ross, R., and P. Bornstein. 1969. The elastic fiber. I. The separation and partial characterization of its macromolecular components. *J. Cell Biol.* **40**: 366.
31. Mandl, I., S. Keller, and M. Levi. 1970. Relationship between the antigenicity and chemical composition of components of elastin digests. *In* Chemistry and Molecular Biology of the Intercellular Matrix. E. Balasz, editor. Academic Press, Inc., New York. **1**: 657.
32. Robert, A. M., Y. Grosgogeat, V. Reverdy, B. Robert, and L. Robert. 1971. Lésions artérielles produites chez le lapin par immunisation avec l'élastine et les glycoprotéines de structure de l'aorte. Etudes biochimiques et morphologiquês. *Atherosclerosis.* **13**: 427.