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Experimental Induction of Atheroarteriosclerosis by the Synergy of Allergic Injury to Arteries and Lipid-Rich Diet

II. Effect of Repeatedly Injected Foreign Protein in Rabbits Fed a Lipid-Rich, Cholesterol-Poor Diet

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Rabbits fed a lipid-rich, cholesterol-poor diet and given concomitant injections of foreign protein, over a period as long as 17 months, developed in their coronary arteries both a) proliferative fibromuscular intimal thickening closely resembling the diffuse intimal thickening that commonly occurs in coronary arteries of man, and b) fatty-proliferative fibromuscular intimal thickening that closely resembles coronary atherosclerosis in man. In contrast, rabbits of another group that were concurrently fed the same diet for as long as 22 months without injections of foreign protein developed changes in arteries of their hearts that resemble neither coronary atherosclerosis nor diffuse intimal thickening in man. Fatty-proliferative changes in aortas of the first group of rabbits are strikingly greater and more closely resemble human aortic atherosclerosis than those in the latter group. In the course of the experiments, the average serum cholesterol was not significantly different in the two groups of rabbits. It was approximately 200 to 250 mg%, which is the average serum cholesterol in adult humans in the United States. These experiments support the hypothesis that the synergy of arterial injury, in particular immunologic injury, and a diet rich in lipid can lead to atherosclerosis in man (Am J Pathol 73:265–300, 1973).

THE HYPOTHESIS that the combined action of injury to man's arteries and his diet can lead to atherosclerosis has been tested

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in this laboratory for many years. In particular, the effect of the combined action of immunologic injury and lipid-rich diet has been investigated.[1-3] We previously reported experiments in which the synergy of allergic injury to arteries and a diet rich in cholesterol caused atherosclerosis in rabbits which in several respects resembles atherosclerosis in man.[1] The animals in those experiments were injected repeatedly with foreign serum protein at intervals of 2 to 3 weeks and were concomitantly fed a dietary supplement of cholesterol for as long as 80 days. The average amount of cholesterol in serum of the animals reached a peak level of approximately 700 mg%. In man the amount of cholesterol in serum is rarely as great as 700 mg%; compared with the arterial injuries induced in rabbits in those experiments, arterial injuries in man are likely to be more protracted or recurrent at longer intervals over a much longer period. Accordingly, it appeared reasonable to hypothesize that atherosclerosis more closely resembling that in man could be induced by more chronic arterial injury in combined action with a diet resulting in cholesterolemia of the same order of magnitude as that in man.

The purpose of this communication is to report the induction of atherosclerosis in rabbits which bears marked similarity to atherosclerosis in man by the combined action of foreign protein injected repeatedly at 4- to 8-week intervals and a lipid-rich, but cholesterol-poor diet fed concomitantly over a period of several to 17 months.

In the course of the experiments, the average serum cholesterol in these animals was approximately 200 to 250 mg%, which is the average serum cholesterol in adult humans in the United States.[4]

Materials and Methods

Rabbits

The total of 174 rabbits, comprising 106 New Zealand reds, 43 grey chinchillas and 25 Dutch-belted rabbits, were obtained from either of two local breeders. The animals had been recently weaned and weighed between 700 and 1000 g. They were examined on arrival and observed for disease for at least 2 weeks before experiments. Only healthy appearing animals were accepted for the experiment. The animals were housed in single cages in which the animal is on a grid of large enough mesh to permit free passage of feces into a removable tray and thus prevent coprophagy.

Diets

During the experiment, 145 rabbits received one of two semisynthetic high fat diets. The composition of these diets, which are modifications of a diet described by Thacker and Brandt,[5] is given in detail in Table 1. In brief, diet I contained, by weight, 24 to 30% protein as casein, 24 to 31% carbohydrate as dextrin, 16 to

Table 1—Composition of Diets

Constituents	Diet I (125 rabbits)	Diet II (20 rabbits)	Vitamin premix provides the following/100 g of diets I and II	
Total lipid	19–25%	24%	Water soluble vitamins	
Hydrogenated vegetable oil*	16–22%	—	Thiamine	0.7 mg
Corn oil†	3%	—	Riboflavin	1.0 mg
Lard‡	—	24%	Calcium pantothenate	1.5 mg
Casein§	28–30%	24%	Pyridoxine	0.7 mg
Dextrin‖	24–31%	31%	Niacin	20.0 mg
Alfalfa meal¶	6%	5%	Choline	100.0 mg
Alphacel‖	6–11%	11%	Betaine	100.0 mg
Macro minerals**	5%	5%	Inositol	10.0 mg
Minor minerals††	0.1%	0.1%	p-aminobenzoic acid	0.2 mg
Water soluble vitamins	see right		Folic acid	0.1 mg
Fat soluble vitamins	see right		Biotin	0.05 mg
			B_{12}	5.0 μg
			Fat soluble vitamins	
			Vitamin A palmitate	666 IU
			Calciferol	0.02 mg
			α Tocopherol	7.50 mg
			Menadione	0.075 mg

* Hydrol, Durkee Foods, Inc, Jamaica, NY
† Mazola, Corn Products Refining Company
‡ Theobold Industries, Long Island City, NY
§ A.N.R.C. High Nitrogen Casein, Sheffield Chemical Co, Norwich, NY
‖ Nutritional Biochemicals, Cleveland, Ohio
¶ Andrew Goetz & Sons, Jericho, NY
** Hawk Oser Salt Mixture, Nutritional Biochemicals, Cleveland, Ohio
†† $FeC_6H_5O_4 \cdot 1\frac{1}{2} H_2O$—453.85; $CuSO_4 \cdot 5H_2O$—28.15; $MnSO_4 \cdot H_2O$—16.50; KI—1.50 g/kg

22% hydrogenated coconut oil, 3% corn oil, 6% alfalfa meal and 6 to 11% alphacel. Diet II contained 27% protein as casein, 31% carbohydrate as dextrin, 24% lard, 5% alfalfa meal, and 11% alphacel. The diets were supplemented with major minerals, minor minerals and vitamins, and were stored at —4 C until immediately prior to feeding.

The amount of cholesterol and plant sterols in the semisynthetic diets I and II were measured.* One-gram aliquots of diets were extracted with petroleum ether following alkaline hydrolysis. The neutral steroids (cholesterol plus plant sterols) were then isolated by thin layer chromatography. Quantitation of cholesterol and plant sterols was achieved by gas-liquid chromatography and correction for loss during extraction and thin-layer chromatography was made by the use of cholesterol-4-^{14}C as an internal standard.

Twenty-nine other rabbits received a lipid-poor commercial diet of Rockland rabbit ration pellets which contains ground whole wheat, sun-cured alfalfa meal, soy bean meal, ground barley and 1% dicalcium phosphate. Periodic analyses have shown that by weight the protein content of this diet has been approximately 17.5%; carbohydrate, 65% and fat, 1.5 to 1.75%

* Dr. Demetrius Pertsemlidis kindly made these determinations in the laboratories of Dr. Edward H. Ahrens at Rockefeller University.

Feeding of Rabbits

The lipid-rich diets were fed as meal and the rations were calculated for each rabbit according to body weight and so adjusted at weekly intervals. After results of preliminary experiments had shown that feeding 30 g diet/kg body weight each day appeared sufficient to maintain normal weight gain, the diet was offered in this amount for the entire experimental period. The meal was weighed and served every other day in pans attached to the side of the cage in such manner that rabbits had access to the food but could not spill it. Any meal not consumed was added to the next ration.

Most of the rabbits in the two groups that were offered lipid-rich diet (groups I and III) were pair-fed. This feeding plan was used to correct for possible differences in amount of food consumed between rabbits in the two groups. Pair-mates in these groups were matched according to breed, sex and body weight at the beginning of the experimental period. If 1 rabbit of a pair did not eat its entire ration on a given day, the following day's ration of its partner in the other experimental group was reduced by the same amount. The remaining rabbits in groups I and III were unpaired and were fed at the rate of 30 g diet/kg of body weight each day.

Rabbits in another group (group II) were fed Rockland rabbit pellets *ad libitum*.

All rabbits were given water *ad libitum*.

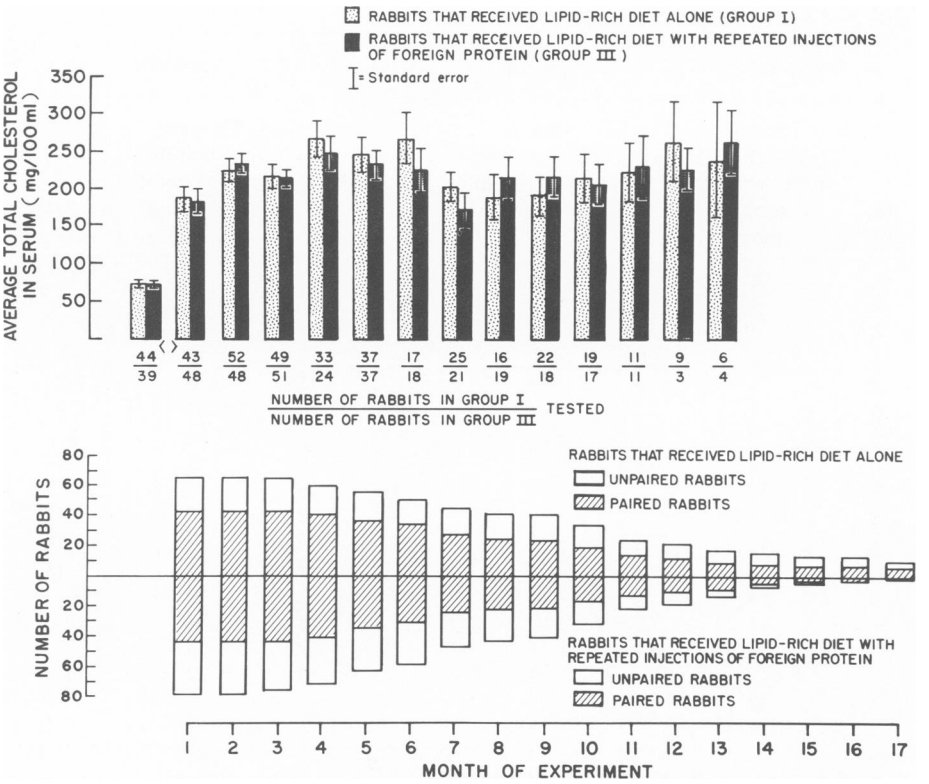
Foreign Serum Proteins

Unpreserved normal horse serum was supplied by Lederle Laboratories, Pearl River, NY, and by the Department of Health of the City of New York. Fresh swine blood was obtained from C. Miller & Co, North Bergen NJ. The blood was allowed to clot in 250-cc centrifuge bottles, and the serum was harvested. The horse and swine sera were lyophilized, stored in sealed bottles and reconstituted to the original volume with distilled water immediately prior to use. Crystalline bovine serum albumin was obtained from Nutritional Biochemicals Corp, Cleveland, Ohio, and dissolved in normal saline prior to injection. The horse and swine sera and solutions of bovine serum albumin were passed through a Seitz filter just before use.

Grouping of Rabbits, Feeding Periods and Injection Schedules

Rabbits were grouped as follows: group I comprised 66 rabbits. They were fed lipid-rich, cholesterol-poor semisynthetic diets for 2 to 22 months (Text-figure 1). Fifty-six rabbits were fed diet I, containing 19 to 25% lipid consisting of 16 to 22% hydrogenated coconut oil and 3% corn oil. Ten rabbits were fed a similar diet, diet II, containing 24% lard. Forty-three of the 66 rabbits in group I were members of pairs, matched with pair-mates in group III according to breed, body weight and sex. The remaining 23 rabbits in group I were unpaired. The animals of group I either died or were sacrificed after eating the diets as indicated in Table 2.

Group II comprised 29 rabbits. These rabbits were fed the lipid-poor Rockland rabbit pellets for 5 to 10 months. During this period they received intravenously five to 10 large injections of sterile horse serum, 10 ml/kg body weight, at intervals of 2 to 8 weeks. Thirty-six hours preceeding each of the last four to nine large injections, 2 desensitizing doses of 1 ml of horse serum were given subcutaneously, each at a different site. An additional 1-ml desensitizing dose of horse serum was given intravenously 12 hours before the large injections. The rabbits died or were sacrificed as shown in Table 2.



TEXT-FIG 1—Upper portion of figure denotes average total cholesterol in serum of rabbits in groups I and III; lower portion shows that the number of rabbits in groups I and III was similar throughout the experiment.

Group III comprised 79 rabbits. They were fed lipid-rich cholesterol-poor semisynthetic diets for 2 to 17 months and were repeatedly injected with foreign serum protein (Text-figure 1). Sixty-nine rabbits were fed diet I. Ten rabbits were fed diet II. Forty-three of the 79 rabbits in group III were members of pairs matched with pair-mates in groups I according to breed, body weight and sex. Thirty-six rabbits were unpaired. Beginning 1 to 2 months after initiation of feeding the lipid-rich diets, the animals received, intravenously, one to thirteen large injections of foreign serum protein at intervals of 4 to 8 weeks. Some rabbits received horse or swine serum, 10 ml/kg of body weight; others received bovine serum albumin, 10 mg/kg. Rabbits were desensitized to foreign serum protein as described for rabbits of group II. The animals were sacrificed or died after eating the lipid-rich diets for the number of months and receiving the number of large injections of foreign serum protein indicated in Table 2.

Collection of Serum and Measurement of Serum Lipids

Approximately one-half of the rabbits were bled from the marginal ear vein every month throughout the experiment; 12-ml samples of blood were collected

Table 2—Feeding Periods and Injection Schedules

Group I		Group II			Group III		
No. rabbits	Time on lipid-rich diet before death or sacrifice (mos)	No. rabbits	Time on lipid-poor diet before death or sacrifice (mos)	No. large injections*	No. rabbits	Time on lipid-rich diet before death or sacrifice (mos)	No. large injections†
1	2	4	2	5	2	2	1
5	3	5	3	6	5	3	1–4
4	4	—	—	—	8	4	2–4
5	5	1	5	—	6	5	2–4
6	6	—	—	—	11	6	4–8
3	7	—	—	—	4	7	6–8
1	8	1	8	9	2	8	6
6	9	18	9–10	10	9	9	6–8
10	10				10	10	8–11
3	11				3	11	7–10
5	12				6	12	8–11
2	13				7	13	9–13
2	14				2	14	9–13
—	—				1	15	10
3	16				2	16	13
2	17				1	17	13
1	18						
7	21						

* Sterile horse serum, 10 ml/kg body weight

† Foreign serum protein: horse or swine serum, or bovine serum albumin, 10 mg/kg body weight

in the morning after the rabbits had fasted overnight. The sera were promptly harvested and stored in small sealed tubes at −20 C until analyzed. If the serum showed evidence of hemolysis, a new specimen was collected.

The total amount of cholesterol in serum was estimated by the method of Abell, Levy, Brodie and Kendall.[6]

The amount of phospholipid in serum was approximated by the method of Abell and Kendall.[7] In this procedure, 1 ml of serum and 15 ml of absolute ethyl alcohol are shaken in a 25-ml volumetric flask, heated to boiling in a water bath and cooled; 7 ml of diethyl ether are added and the mixture is brought to 25 ml with absolute ethyl alcohol, shaken and filtered. Fifteen milliliters of filtrate are placed in a glass tube and completely evaporated. To the residue, 0.1 ml of 0.5 N HCl in 50% ethanol and then 15 ml of petroleum ether are added and the tube is vigorously shaken. The contents are allowed to clear by standing several hours; 0.5 ml of the petroleum ether extract is pipetted into a heavy walled pyrex test tube previously calibrated at 10 ml, and the extract evaporated to dryness. One milliliter of 10 N H_2SO_4 is added and the contents are digested over an open flame until the solution is homogeneously black. Two drops of 30% hydrogen peroxide (Merck, Superoxol®) are added. The tube is again heated until the contents become colorless, and then all of the water and hydrogen peroxide is boiled off by further heating. The tube is cooled and 5 ml

of water are added. One milliliter of a molybdate reagent* is added and the tube shaken; 0.4 ml of an aminonaphtholsulfonic acid reagent† is added and the tube shaken again. The tube is heated in a boiling water bath for 10 to 15 minutes, cooled and made up to 10 ml volume with distilled water. Optical density was read in a Beckman DB Spectrophotometer at 660 mμ using a sulfuric acid hydrogen peroxide blank as a reference. Solutions of potassium dihydrogen phosphate were used to prepare 5 to 15 gamma phosphorus standards. Serum phospholipid was expressed as milligrams of lecithin.

The amount of triclyceride in serum was estimated according to the method of Van Handel and Zilversmit.[8]

Necropsies

Sixty-five rabbits died and 109 were sacrificed. The former were mostly members of goups II and III and died in anaphylactic shock or with acute serum sickness following intravenous injection of foreign serum protein. Animals were sacrificed by intravenous injection of sodium pentobarbital given rapidly. Necropsies were performed on all rabbits. The hearts and aortas were removed and fixed in buffered 10% formalin. Multiple small blocks of tissue were removed from the lungs; liver; gall bladder; spleen; pancreas; skeletal muscle, including portions of major arteries supplying the extremities; stomach; small intestine and attached mesentery; large intestine; kidneys; adrenals; thyroid, including adjacent carotid arteries; testes; ovaries and brain. Some blocks of tissue were fixed in Zenker's-5% acetic acid and others in buffered 10% formalin.

Dissection of Aortas and Grading of Grossly Visible Aortic Changes

The aorta of each animal was opened in entirety, from the aortic valve to the origin of the common iliac arteries, and the inner surface examined. All aortas showing the spontaneously occurring degenerative changes long known to occur in some rabbits were excluded from the analysis to be described. Of the remaining samples of 148 aortas, 61 were from rabbits that received a lipid-rich diet without injections of foreign serum protein (group I), 29 from rabbits that received injections of horse serum and lipid-poor diet (group II), and 58 from rabbits that received a lipid-rich diet and concomitant injections of horse serum (group III).

To evaluate as accurately as possible the overall fatty change in aortas, each of the following three portions of aorta were considered separately: the ascending portion (from aortic valve to just distal to the origin of the brachiocephalic vessels), the descending thoracic portion, and the abdominal portion. Both at the time of necropsy and subsequently, after fixation, the amount of grossly visible fatty change in the lining of the three portions of aortas was graded by two persons, one independent of the other and without knowledge of the experimental group to which the rabbit belonged. Those portions with no grossly visible fatty change were graded as 0, those with small areas of fatty change located primarily near the origin of branches as +, those with larger areas of fatty change located primarily near the origin of branches as ++, those with areas of fatty change involving more than 30% of the luminal surface as +++,

* Molybdate reagent: 84 ml of concentrated H_2SO_4 are added to 300 ml of distilled water; 25 g of ammonium molybdate are dissolved in 200 ml of water. The two solutions are mixed and diluted with distilled water to 1000 ml.

† Aminonaphtholsulfonic acid reagent: 30 g of reagent grade $NaHSO_3$ and 1 g of $Na_2SO_3 \cdot 7H_2O$ are dissolved in 200 ml of distilled water; 0.5 g of 1-amino-2-naphthol-4-sulfonic acid are added, and the solution filtered and stored in a dark bottle.

and those portions with areas of fatty change involving nearly the entire luminal surface as ++++. The total aortic score for any of the three segments of aorta is the sum of the scores for that segment of all aortas of group I or group III that were examined. The average aortic score is the total aortic score divided by the total number of aortas so analyzed in any experimental group. The degree of fatty change was simplified to three categories: those with no change or slight fatty change (0 to +), those with moderate change (++) and those with marked change (+++ to ++++).

Preparation of Tissues for Analysis of Arterial Changes by Light Microscopy

Hearts of rabbits in all groups were dissected in a uniform manner. The entire heart was divided into ten to twelve 3- to 4-mm blocks. One or more blocks of the following were always obtained: a) right ventricular wall, interventricular septum, right noncoronary cusp of the aortic valve, pulmonary valve, base of the aorta, and pulmonary artery; b) nonseptal wall of the right ventricle, tricuspid valve and right atrium; c) anterior lateral wall of the left ventricle, anterior papillary muscle, and mitral valve; d) left venticular wall, posterior papillary muscle, mitral valve, and adjacent left atrial wall and e) left ventricular wall, left coronary cusp of aortic valve, and base of the aorta. Blocks from the right ventricle always included portions of the right main coronary artery; the first group of blocks usually included a portion of this artery adjacent to and including its ostium. Blocks from left ventricular tissue often included the left coronary artery and its major branches.

A block of each of the following segments of aorta was obtained: a) proximal portion of the aorta extending from just distal to the aortic valve to the arch of the aorta; b) arch of the aorta, including the ostia and proximal portions of the brachiocephalic vessels; c) descending portion of the thoracic aorta, including the ostia of many intercostal branches of the aorta; d) proximal portion of the abdominal aorta, including the ostia and proximal portions of the mesenteric and celiac arteries; e) aorta at the level of the renal arteries, which always included the ostia and usually the proximal portions of both renal arteries; f) distal abdominal aorta extending from the renal arteries to and including a portion of the inferior mesenteric artery and g) distal abdominal aorta just proximal to the bifurcation. Blocks of hearts, aortas and other organs were embedded in paraffin and sections were cut at 5 μ and stained routinely with hematoxylin and eosin. Many sections were also stained with Weigert–hematoxylin and eosin.

Comparable numbers of sections of hearts and aortas of rabbits of all experimental groups were examined with the light microscope. For purposes of analysis, coronary arterial lesions were classified according to size of artery involved (large, medium or small) and histologic character (fatty, proliferative or fatty-proliferative), as described previously.[1] Main arteries and major branches were classified as large. Intramyocardial arteries that were not major branches were classified as either medium or small.

Changes in the various portions of the aortas were examined and compared by two persons without knowledge of the treatment the rabbits received. The aortic changes were scored quantitatively according to the thickness of the altered intimal and subintimal tissue and qualitatively according to the type of histologic change. Sections of aorta with no visible microscopic change were scored as 0, those with medial change but no appreciable intimal change as ±, those with slight intimal thickening as +, those with moderate intimal thickening as ++, and those with marked intimal thickening as +++. Qualitatively, the changes in the aortas fell into three descriptive categories: fatty, proliferative, and fatty-proliferative. In further analysis, a total aortic score and average aortic score

were used as indices of the severity of aortic change. The total aortic score for any segment of the aorta is the sum of the scores of all lesions of one histologic type in that segment of all aortas of group I or group III that were examined. The average aortic score is the total aortic score divided by the total number of aortas so analyzed in any experimental group.

Results

Sterol Content of the Lipid-Rich Diets

Analysis of the semisynthetic diets indicated that diet I, in which 16 to 22% hydrogenated coconut oil and 3% corn oil were used as sources of fat, contained 29.7 μg of cholesterol and 343 μg of plant sterols/g diet. Diet II, in which 24% lard was used as a fat source, contained 166 μg of cholesterol and 111 μg of plant sterols per gram of diet. Thus on a percentage basis, diet I contained 0.04% total neutral steroids and 0.003% cholesterol, and diet II contained 0.027% total neutral steroids and 0.016% cholesterol.

Feeding of the Lipid-Rich Diets

The lipid-rich diets were fed in the form of meal. Rabbits usually required a period of approximately 1 month to adapt to eating food in this form. Only recently weaned rabbits were used in these experiments, and they learned to eat the meal. It had been found that older rabbits, previously fed commercial diets in pellet form for long periods of time, adapted poorly and some refused the diet or ate only small quantities.

Rabbits were fed 30 g diet/kg body weight each day, and most of them consumed nearly the entire quantity. Animals gained weight normally and exhibited no evidence of dietary deficiency.

Serum Lipids

In groups I and III, as recorded in Text-figure 1, the average total cholesterol in serum increased similarly during the first 3 months of feeding of the lipid-rich diets and remained similar for the duration of the experiment. The average total cholesterol for group I increased from an initial value of 72 mg/100 ml to between 187 and 268 mg/100 ml, and that for group III from an initial value of 73 mg/100 ml to between 171 and 262 mg/100 ml. Similarly, average phospholipids in group I increased from an initial value of 77 mg/100 ml to between 159 and 232 mg/100 ml, and in group III from an initial value of 73 mg/100 ml to between 152 and 196 mg/100 ml. Serum triglycerides in rabbits of groups I and III did not increase

significantly during the course of the experiment. In group I, the average baseline value for serum triglycerides was 102 mg/100 ml and the range during the period of feeding the lipid-rich diets was 70 mg/100 ml to 140 mg/100 ml; in group III the average baseline value was 88 mg/100 ml, and the range during the period of feeding these diets was 57 to 133 mg/100 ml.

In the very large majority of animals in group II, there was no increase in the total serum cholesterol during the experiment. In a few rabbits there was a slight increase. Serum phospholipids and triglycerides in rabbits of group II were not estimated.

Arterial Lesions

Arterial lesions developed in rabbits of all groups. Their distribution and qualitative characteristics varied with treatment. In group I there were numerous lesions in coronary and pulmonary arteries and aortas, but only very occasionally were lesions found in other arterial beds. In groups II and III there were also numerous lesions in coronary and pulmonary arteries and aortas, and in addition there were numerous lesions in many other arterial beds, including mesenteric, splenic, gastric, renal, femoral, carotid and subclavian arteries.

In group I the majority of arterial lesions were fatty and, except for those in the aortas and pulmonary arteries, they occurred principally in arteries of small to medium size. Proliferative arterial lesions occurred only in the heart and were limited almost entirely to arteries of small and medium size.

In group II the arterial changes were virtually all proliferative lesions without fatty change. In contrast with those of group I, they occurred in arteries of all sizes and resembled, some closely, human arteriosclerosis without fatty change (diffuse intimal thickening).

In group III the arterial changes were virtually all proliferative or fatty-proliferative lesions. Like those in group II they occurred in arteries of all sizes. A large number of them were like those of human arteriosclerosis without fatty change (diffuse intimal thickening). Many others closely resembled atherosclerosis in man.

The changes in coronary arteries and aortas are reported in detail.

Coronary Arterial Lesions

In group I, as shown in Table 3, 71% of the coronary arterial lesions occurred in small and 22% in medium arteries. Only occasionally (7%) were lesions found in large arteries, and these in the majority were at the coronary ostia, the walls of which are histologically like

Table 3—Incidence, Distribution and Histologic Types of Coronary Arterial Lesions

Group and regimen	No. rabbits with arterial lesions (% of rabbits)	No. arterial lesions examined	Average No. arterial lesions/rabbit	Distribution of arterial lesions (% of lesions)			Incidence of histologic types of arterial lesions (% of lesions)		
				Large arteries	Medium arteries	Small arteries	Fatty	Proliferative	Fatty-proliferative
Group I Received lipid-rich diet alone (N = 66)	46 (71%)	421	6.5	31 (7%)	93 (22%)	297 (71%)	219 (52%)	160 (38%)	42 (10%)
Group II Received lipid-poor diet with repeated injections of foreign protein (N = 29)	29 (100%)	658	22.7	165 (25%)	195 (30%)	298 (45%)	(0%)	645 (98%)	13 (2%)
Group III Received lipid-rich diet with repeated injections of foreign protein (N = 79)	77 (96%)	1205	15.3	290 (24%)	349 (29%)	566 (47%)	24 (2%)	904 (75%)	277 (23%)

The percent of rabbits with arterial lesions and the average number of lesions per rabbit was greater in the two groups injected with foreign protein. In the group that received lipid-rich diet alone, the majority of lesions were fatty and occurred in small and medium arteries, and only a small number were found in large arteries. In contrast, in the two groups that received foreign protein, the vast majority of lesions were proliferative or fatty-proliferative, were distributed similarly throughout arteries of all sizes, and many were in large arteries.

those of the aorta. The majority of all of the arterial changes found in this group and the majority of the small number of lesions found in large coronary arteries proper were fatty. They were characterized by accumulation of lipid in intimal and medial cells and extracellularly with very little or no cellular proliferation (Figure 29). Most of the remaining arterial changes were proliferative with no fatty component and almost entirely limited to small and medium arteries. They were segmental, comprised proliferated intimal and medial cells, and exhibited hyperplastic musculoelastic intimal change and focal fragmentation and/or reduplication of internal elastic membrane (Figure 30). We have concluded that at least the large majority of the proliferative lesions in group I occurred spontaneously and were unrelated to our experiment because of the following data. Spontaneously occurring identical proliferative changes, in similar incidence and again almost entirely limited to small and medium arteries, were found in hearts of many control rabbits, not subjected to experiment, which had come from the same dealers who provided the rabbits in our experiments.[9] In a relatively small number of proliferative lesions in group I, lipid had accumulated in intimal cells.

When animals in group I were grouped according to level of serum cholesterol, the average number of fatty arterial lesions per rabbit increased with increase in the average amount of cholesterol in serum. Thus, 46 rabbits with average serum cholesterol less than 250 mg% had, together, 79 fatty lesions in coronary arteries, or an average of 1.7 lesions per rabbit; while 18 rabbits with average cholesterol greater than 250 mg% had 117 fatty lesions in coronary arteries, or an average of 6.5 lesions per rabbit. This difference is significant ($.02 > P > .01$, as determined by Student's t test). The number of proliferative arterial lesions was not related to the degree of hypercholesterolemia. Twenty-five rabbits with average cholesterol levels of less than 150 mg% had 82 proliferative arterial lesions or 3.2 lesions per rabbit; while 21 rabbits with average cholesterol between 150 and 250 mg% had 49 arterial lesions or 2.3 lesions per rabbit. Eighteen rabbits with average cholesterol greater than 250 mg% had 76 lesions or 4.2 per rabbit.

Rabbits of group I that were fed the lard diet (diet II) died or were sacrificed no later than 6 months after feeding of this diet began. The remainder of group I were fed the hydrogenated coconut oil diet (diet I), and they died or were sacrificed at times ranging from several weeks to 22 months after feeding of the diet began. The average number of fatty lesions in coronary arteries of rabbits fed

diet I increased within the first 6 months of the experiment. Thereafter, no increase occurred. In contrast, the average number of proliferative arterial lesions did not increase either during the first 6 months of the experiment or afterward, which is in harmony with our conclusion stated above that at least the large majority of these proliferative lesions occurred spontaneously and were unrelated to the experiment.

In group II, lesions were found throughout the coronary arterial tree. Twenty-five percent were in large arteries, 30% in medium and 45% in small arteries. The vast majority (98%) were proliferative lesions. These changes were segmental and comprised musculoelastic hyperplastic or hyaline intimal change, focally fragmented and/or reduplicated internal elastic membrane, thinned and scarred media, and cellular proliferative and infiltrative change and fibrosis in the adventitia. Most of these changes are illustrated in Figure 1. Note the close resemblance of these changes to those in the coronary arteries of a 12-year-old girl (Figure 2) and a 6-year-old girl (Figure 4). Some proliferating cells in the intima, media and adventitia of the coronary arteries of group II contained nuclei with caterpillar-like chromatin pattern or owl eye appearance.[1,10] Rarely, in a proliferative lesion, lipid had accumulated in some intimal cells.

In group III, lesions were found throughout the coronary arterial tree, and their distribution among arteries of various size was identical to that in group II (Table 3). As compared with the arterial lesions of group I, those of group III differed significantly as follows: a) the proportion of rabbits that developed lesions was greater in group III, b) there was different distribution of lesions acording to artery size and c) there was different incidence of histologic types (in all three cases X^2_y, $P \ll .001$). The arterial changes in group III were of three types: fatty, proliferaive and fatty–proliferaive. Only very occasional (2%) lesions were fatty. The majority (75%) were proliferative. The characteristics of both the fatty and the proliferative lesions are described above. The remainder (23%) of the arterial lesions in group III were fatty-proliferative. They were similar to those in group II but were modified by fatty changes. The average number of lesions in large coronary arteries of group III was almost eight times greater than that in group I (Table 4). Of the lesions of large coronary arteries, the majority were fatty in group I, whereas they were virtually all proliferative or fatty-proliferaive lesions in group III. This difference is significant (X^2, $P \ll .01$). In large coronary arteries of group III, the average number of proliferative lesions was twenty times

Table 4—Incidence and Histologic Types of Lesions of Large Coronary Arteries

Group and regimen	No. rabbits with lesions of large coronary arteries (% of rabbits)	No. lesions of large coronary arteries	Average No. arterial lesions/rabbit	Incidence of histologic types of arterial lesions (% of lesions)		
				Fatty	Proliferative	Fatty-proliferative
Group I Received lipid-rich diet alone (N = 66)	19 (29%)	31*	.47	20 (64%)	7 (23%)	4 (13%)
Group III Received lipid-rich diet with repeated injections of foreign protein (N = 79)	59 (74%)	290†	3.7	3 (1%)	168 (58%)	119 (41%)

* The majority of these lesions are at coronary ostia, not in coronary arteries proper.

† The large majority of these are in coronary arteries proper, a minority are at coronary ostia.

The number of rabbits with lesions in large coronary arteries in group III is three times that in group I. The average number of lesions in large coronaries in group III is eight times that in group I. From data in the table it may be calculated that the average number of proliferative lesions in large coronaries of group III was twenty times greater and the average number of fatty-proliferative lesions twenty five times greater than in group I.

greater and the average number of fatty-proliferative lesions was twenty-five times greater than those, respectively, in large coronary arteries of group I.

The fatty-proliferative arterial changes in group III were segmental and included proliferated intimal and medial cells, focally fragmented and reduplicated internal elastic membrane, lipid in both intimal and medial cells, fatty-hyaline intimal change, accellular pools of lipid and cholesterol clefts deep in the intima and media with overlying fibromuscular caps (Figures 3, 5, 7, 8, 11–13, 15, 17, 20, 21 and 23). Note the striking similarity of these various changes to those in human coronary atherosclerosis shown in Figures 4, 6, 9, 10, 14, 16, 18, 21, and 22. Thickened intima was often vascularized (Figure 23), as in human coronary atherosclerosis (Figure 22). Segmental medial necrosis or scarring with thinning of the media was sometimes observed (Figure 17), as occurs in human coronary atherosclerosis (Figure 18). Other changes encountered were increased numbers of small blood vessels in the media and adventitia and cellular proliferative and infiltrative change and fibrosis in the adventitia. Some intimal and me-

dial cells, including foam cells, contained nuclei with caterpillar-like chromatin pattern or owl eye appearance. Rarely, fatty-proliferative intimal thickening was accompanied by a small deposit of calcium (Figure 24), as occurs in human atherosclerosis. Occasionally, a thrombus was found attached to altered intima (Figure 26).

In contrast with group I, the average number of fatty arterial lesions per rabbit in group III did not increase with increase in serum cholesterol. Similarly, the average number of arterial lesions per rabbit of all histologic types did not increase with increase in serum cholesterol. Thus, 46 rabbits with average total cholesterol less than 250 mg% had 20.4 arterial lesions per rabbit; while 17 rabbits with average total cholesterol greater than 250 mg% had 9.28 arterial lesions per rabbit. The number of proliferative arterial lesions was proportional to the total number of arterial lesions. The proportion of the arterial lesions which were fatty-proliferative was greater in rabbits with cholesterol greater than 250 mg%. Forty-two percent of coronary arterial lesions were fatty-proliferative in a group of rabbits with average serum cholesterol greater than 250 mg%, while only 15% were fatty proliferative in a group with average serum cholesterol less than 250 mg%.

Rabbits of group III that were fed the lard diet died or were sacrificed no later than 6 months after feeding of the diet began. The remainder of group III that were fed the hydrogenated coconut oil diet died or were sacrificed at various times ranging from several weeks to 17 months after feeding of the diet began. Although the average number of coronary arterial lesions increased during the first 6 months of the experiment to reach a figure of 14.9 lesions per rabbit in animals examined between the sixth and thirteenth months, the number of arterial lesions in animals after the thirteenth month was found not to have increased further, but in fact, was found to have decreased to 6.7 lesions per rabbit. The number of proliferative arterial lesions was in proportion to the total number of arterial lesions. The number of fatty-proliferative arterial lesions increased within the first 6 months of the experiment. Thereafter, no increase was found to have occurred. However, the ratio of fatty-proliferative lesions to total number of lesions increased approximately threefold with time. The increase in this ratio was particularly striking in the case of large coronary arteries.

Aortic Lesions

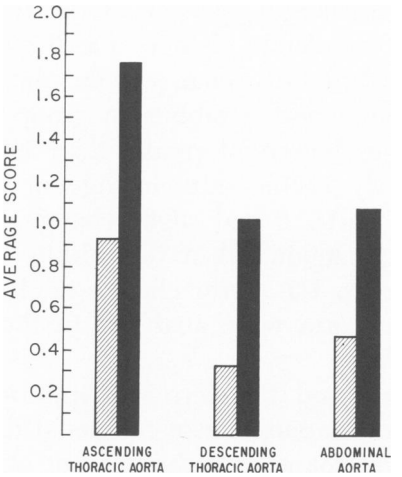
In the very large majority of rabbits of group II there was no grossly visible change in the lining of the aortas. The lining of a small number

of aortas contained a small number of grossly visible, small yellow-white elevated plaques, the majority of which occurred in the thoracic aorta near the origin of branches. Microscopically, changes were found in one or more of all the segments of the aorta, and they were usually slight, but were more marked near the origin of branches. These alterations comprised degenerative changes, including necrosis of subintimal smooth muscle, fragmented elastic lamellae, and proliferated intimal and subintimal cells. The most marked example of these changes that we encountered is shown in Figure 32. Following subsequent repair, the thickened or new intima appeared in considerable part to represent formerly injured and then replaced subintimal media. Similar replacement of media in human aortic atherosclerosis also appears likely (Figure 34).

In the majority of rabbits fed a lipid-rich diet (groups I and III), either no change or slight change in the lining of the aortas was seen grossly. The remaining rabbits developed either a moderate or marked amount of grossly visible aortic change in the form of yellow-white streaks and plaques. The ascending portion of the thoracic aorta was most often involved and exhibited the most extensive changes. As a group, arotas of rabbits fed a lipid-rich diet and concomitantly injected with foreign serum protein (group III) exhibited much greater change than did those of animals fed the same diet without injections of foreign serum protein (group I). This is shown in Text-figure 2, and the difference for all three segments of aorta is significant (Student's t test, group I vs group III, ascending aorta, $P < .001$; descending thoracic aorta, $P < .001$; abdominal aorta, $.05 \gg P > .02$.

Moreover, of those animals that developed grossly visible marked change in the aorta, the very large majority were members of group III (Table 5). Thus, of 26 rabbits that developed marked fatty change in the ascending portion of the thoracic aorta, 20 or 77% were in group III that were fed a lipid-rich diet and concomitantly received repeated injections of foreign serum protein. Of the 10 rabbits that developed marked fatty change in the descending portion of the thoracic aorta, all were in group III; of the 11 rabbits that developed marked fatty change in the abdominal portion of the aorta, 10 were in group III (in all three instances these differences are significant: X^2 test, $.005 > P > .001$).

In general, the amount of fatty change in the various segments of aortas of rabbits in both groups I and III was related to the amount of cholesterol in the serum. However, even among rabbits of groups I and III with similar range of serum cholesterol, the amount of fatty



TEXT-FIG 2—Amount of grossly visible streaks and plaques in all three segments of aortas of animals in group I (61 rabbits that received lipid-rich diet alone, *hatched columns*) and group III (58 rabbits that received lipid-rich diet with repeated injections of foreign protein, *solid columns*).

change was greater in group III. For example, 21 rabbits in group I and 17 rabbits in group III had an average serum cholesterol less than 150 mg%. Of these 38 rabbits, 36 exhibited either no grossly visible fatty change or only slight change in the descending portion of the thoracic aorta. The remaining 2 rabbits exhibited moderate change, and they were in group III. Thirty-one rabbits in group I and 36 rabbits in group III had an average serum cholesterol between 150 and 300 mg%. Of these 67 rabbits, 38 exhibited no grossly visible

Table 5—Aortic Atherosclerosis

Group and regimen	No. aortas examined	Number rabbits with marked atherosclerosis of aorta		
		Ascending thoracic aorta	Descending thoracic aorta	Abdominal aorta
Group I Received lipid-rich diet alone (N = 66)	61	6	0	1
Group III Received lipid-rich diet with repeated injections of foreign protein (N = 79)	58	20	10	10

The vast majority of animals that developed marked atherosclerotic change in the aorta were in group III.

fatty change in the descending portion of the thoracic aorta, 17 had only slight change, 6 moderate change, and 6 marked change. All of the rabbits with marked fatty change were members of group III. Nine rabbits in group I and 5 rabbits in group III had an average amount of total serum cholesterol greater than 300 mg%. Two of these rabbits had no grossly visible fatty change in the descending portion of the thoracic aorta, 6 had slight change, 2 moderate change, and 4 marked change. Again, all of the rabbits with marked change were members of group III. Fatty change in the ascending thoracic aorta and abdominal aorta were analyzed in the same manner, and the results were similar.

Rabbits of groups I and III were fed lipid-rich diets for varying periods of time. From examination of rabbits at different times during the experiment, it was found that the amount of fatty change in the various aortic segments increased to reach a maximum between 6 and 13 months. It is notable that after 13 months there was no further increase. Group III had greater fatty change in all aortic segments throughout the course of the experiments.

Microscopically, aortic lesions in groups I and III were fatty or fatty-proliferative. In the fatty lesions, intimal cells and subjacent medial smooth muscle cells contained a large amount of lipid (Figure 31). In fatty–proliferative lesions, there were degenerative changes, including fatty change and necrosis, of subintimal smooth muscle and intimal cells, fragmented elastic lamellae, and proliferated intimal and subintimal cells (Figure 33). These changes resembled those of human aortic atherosclerosis (Figure 34). In the rabbit lesions it is usually evident and in human atherosclerosis it is sometimes apparent that thickened intima in considerable part represents formerly altered and replaced subintimal media. In a few animals of group III more advanced fatty-proliferative atherosclerosis of the aorta with fatty-hyaline intimal and medial change and pools of lipid deep in the arterial wall (Figures 35 and 37) was encountered; and these changes strikingly resembled aortic atherosclerosis in man (Figures 36 and 38). Such changes were not encountered in animals of groups I or II.

The amount of atherosclerotic change, as determined by the thickness of the lesions, was nearly twice as great in thoracic and abdominal segments of aortas in group III as compared with group I. This difference is significant (Student's t test, $.01 \gg P > .001$, for both thoracic and abdominal segments). This difference was due to a more than fourfold greater amount of fatty-proliferative change in aortas of group III.

Lesions of Other Muscular Arteries

In rabbits of groups II and III arterial lesions of mesenteric, splenic, gastric, renal, femoral, carotid and subclavian arteries were qualitatively similar to those in the coronary arteries. Repeated injections of foreign protein did not cause lesions in cerebral arteries, nor were cerebral arterial lesions encountered in group III. Animals of group I also failed to develop lesions in cerebral arteries.

Lesions of Cardiac Valves

In many rabbits of groups I and III there were grossly visible fatty lesions in the cusps of aortic valves and leaflets of mitral valves. Although not quantitated, it appears that the amount of fatty change in cardiac valves was appreciably greater in group III. Occasionally in animals of group III thrombi were found attached to altered mitral (Figure 27) or aortic valve annuli, or there were organized thrombi in valve pockets (Figure 28).

Discussion

In those groups of rabbits that were fed lipid-rich, cholesterol-poor diet, either with (group III) or without (group I) repeated injections of foreign protein, the amount of cholesterol in serum increased from a preexperiment average value of approximately 70 mg% to an average value for the course of the experiment of approximately 200 to 250 mg%, which is the average amount of cholesterol in serum of adult humans in the United States. In only a few of the rabbits that were fed a lipid-poor diet with repeated injections of foreign protein (group II) was there an increase in serum cholesterol, and this increase was slight.

In all three groups of animals, there were lesions in coronary and pulmonary arteries and aortas. In groups II and III there were also numerous lesions in many other arterial beds, whereas only very occasional lesions were encountered in these other arterial beds in group I. The arteries of the heart will be discussed first. In group I, the vast majority (93%) of lesions were in small or medium arteries; neither these nor the small number of lesions found in large coronary arteries resemble coronary atherosclerosis in man. In group II the lesions comprised proliferative fibromuscular or sometimes hyaline intimal changes in arteries of all sizes, often fragmented and/or reduplicated internal elastic membrane, and degenerative medial changes. As shown in comparative illustrations, many of these lesions closely resemble the arteriosclerosis that commonly occurs in human

coronary arteries without manifest lipid, often referred to as diffuse intimal thickening.

In group III the vast majority (98%) of lesions comprised proliferative or fatty-proliferative fibromuscular or hyaline intimal changes, often fragmented and/or reduplicated internal elastic membrane, and degenerative medial changes. In their proliferative character and distribution these lesions were very similar to those of group II. Inasmuch as rabbits of both groups II and III were injected in the same manner with foreign serum protein, it is reasonable to infer that a) in group II, in the absence of hyperlipemia, proliferative lesions without fatty change developed at sites of allergic injury to arteries; whereas b) in group III, in the presence of hyperlipemia, fatty-proliferative lesions evolved at some of the sites of allergic injury to arteries.

The fatty-proliferative fibromuscular and fatty-hyaline lesions of group III closely resemble atherosclerosis in man, as shown in comparative illustrations. These changes include focally fragmented and reduplicated internal elastic membrane, proliferated intimal and medial cells, some of them lipid-filled foam cells scattered throughout or clustered, regions of fatty-hyaline change with few or no manifest elastic fibers in these regions, pooled lipid and cholesterol clefts deep in intima and media with overlying fibrocellular cap or jacket, and vascularization. Other changes in both the rabbit and human atherosclerosis are segmental necrosis, scarring and thinning of the media; and cellular proliferative and infiltrative change and fibrosis in adventitia. Occasionally, thrombi were found attached to altered arterial intima. Rarely a small deposit of calcium was found deep in thickened intima of a rabbit artery.

From data acquired previously[1] and in the present experiments, it is reasonable to conclude that the fatty-proliferative fibromuscular and fatty-hyaline arterial lesions of group III that closely resemble human atherosclerosis began and recurred as acute reactions to allergic injury that were modified by insudated blood-borne elements, including lipid. On the other hand, in more recent experiments in this laboratory, it was shown that immunologically but previously induced arterial intimal thickening, closely resembling diffuse intimal thickening in man, later accumulated lipid preferentially in the presence of hypercholesterolemia and evolved as atherosclerosis.[11] Thus, in some of the fatty-proliferative lesions in the presently reported experiments, lipid may have similarly accumulated preferentially in arterial intima thickened previously by reaction to immunologic injury.

The amount of grossly visible yellow-white streaks and plaques that developed in aortas of groups I and III was strikingly greater in group III. Microscopically, the amount of aortic change, as measured by thickness of plaques, was significantly greater in group III, due to a fourfold greater amount of fatty-proliferative intimal and medial change. Only slight proliferative change was found in a small number of aortas of group II. Inasmuch as rabbits of both groups I and III were similarly fed lipid-rich diet, but group III in addition concomitantly received repeated injections of foreign protein, it is reasonable to infer that the significantly greater amount of fatty-proliferative change in aortas of group III was due to the combined action of immunologic injury and lipid-rich diet. Furthermore, advanced fatty-proliferative atherosclerosis closely resembling human aortic atherosclerosis, as here illustrated, occurred in group III but not in groups I or II.

It is becoming increasingly evident that injury to the arterial wall is probably a primary causative factor in atherosclerosis.[1,12–19] Rössle [14] hypothesized and Haust [19] recently supported the view that atherosclerosis is in essence an inflammatory process in which arterial injury, local reaction and repair occur in sequence with secondary degenerative phenomena. We similarly look upon atherosclerosis as a condition in which inflammation and degenerative processes occur in reaction to various injuries and are modified in varying degree by insudated blood-borne elements, including lipid in particular.

In searching to find those causes of arterial injury which lead to atherosclerosis in man, immunologic injury comes to mind. It has been proposed that allergy to antigens in infecting microorganisms and vaccines, foreign serum, antibiotics and other drugs, tobacco, foodstuffs, the individual's own tissues and other antigens, may be causative of much arterial disease which can evolve as atherosclerosis.[1,20] In testing this hypothesis, we have produced by means of chronic immunologic injury and lipid-rich diet an animal model of atherosclerosis which in several important respects resembles atherosclerosis in man. First of all, immunologic reactions occur commonly in the human population. Secondly, the hypercholesterolemia of the animal model is of the same order of magnitude as that of adult humans in the United States. Thirdly, arterial lesions induced by repeated injections of foreign serum protein in rabbits concomitantly fed a lipid-rich diet are histologically very similar to those of human atherosclerosis. Marked involvement of major coronary arteries is a prominent feature of both atherosclerosis in man and in the rabbits that received

lipid-rich diet and repeated injections of foreign serum. In contrast, in the rabbits that received the lipid-rich diet alone, there was little involvement of major coronary arteries, and neither these lesions nor those that developed in small and medium coronary arteries of these animals resembled atherosclerosis in man.

The primary purpose of including the illustrated cases of human coronary and aortic atherosclerosis in this report is to provide human material for morphologic comparison with the experimentally induced atherosclerosis. However, certain comments about the nature of the human cases appear to be pertinent to the experimental investigation reported. In 8 of these 10 cases, acute or chronic rheumatic cardiovascular disease, systemic lupus erythematosus or rheumatoid disease was the principal disease. In the causation of all three of these diseases, immunopathologic factors are very probably of great importance.[21–36] Furthermore, it has long been known that injury to the aorta, especially the thoracic portion, and to coronary and other arteries occurs in rheumatic fever and can lead to sclerotic change in these arteries.[27–31] In some of these arteries, as in the sclerotic cardiac valves of these rheumatic subjects, atherosclerosis may evolve.[32–34] Moreover, injury to large coronary and other arteries in systemic lupus erythematosus,[35–37] and to the aorta and other large arteries in rheumatoid arthritis [38,39] can lead to sclerotic change in these vessels.

The occurrence of coronary or aortic atherosclerosis with systemic diseases in which immunopathologic factors probably play a major role may be fortuitous. It is possible, however, that repeated or protracted immunologic injury and local changes in reaction to the injury favored repeated or protracted deposition and accumulation of blood-borne elements, in particular lipid, in the arterial walls, and thus lead to atherosclerosis. This possibility is supported by results of the experimental investigations here reported.

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Legends for Figures

Fig 1—Arteriosclerosis, without manifest lipid, of the left coronary artery of rabbit 11570 (group II) that received lipid-poor commercial rabbit ration and 10 injections of horse serum at intervals of 2 to 8 weeks. Total cholesterol in serum within normal range. Concentric musculoelastic intimal thickening with fragmentation and reduplication of the internal elastic membrane. Note the marked similarity to the arteriosclerosis of the human coronary artery shown in Figure 2 (Weigert-hematoxylin and eosin, × 78).

Fig 2—Arteriosclerosis, without manifest lipid, of the left anterior descending coronary artery of a 12-year-old girl who died of chronic active rheumatic heart disease with congestive heart failure (autopsy 12873, The New York Hospital). Serum cholesterol content not known. Concentric musculoelastic intimal thickening with straightening, fragmentation, and reduplication of the internal elastic membrane. Note the marked similarity to the arteriosclerosis of the rabbit coronary artery shown in Figure 1 (Weigert-hematoxylin and eosin, × 60).

Fig 3—Arteriosclerosis, with slight atherosclerotic change, of the left coronary artery of rabbit 10906 (group III) that received semisynthetic lipid-rich diet II and 6 injections of horse serum over a period of 7 months. Average total cholesterol in serum was 213 mg%. Musculoelastic intimal thickening with straightening, fragmentation, and reduplication of the internal elastic membrane. Deep in the intima, close to the internal elastic membrane are small deposits of lipid. Note the marked similarity to the human coronary arteriosclerosis shown in Figure 4 (Weigert-hematoxylin and eosin, × 120).

Fig 4—Arteriosclerosis, with slight atherosclerotic change, of the left circumflex coronary artery of a 6-year-old boy who died during the second of two known attacks of rheumatic fever (this section, from a heart acquired outside this medical center, is one of the series No. 71 in the pathology teaching set of slides at Cornell University Medical College). Serum cholesterol content not known. Musculoelastic intimal thickening with fragmentation and reduplication of the internal elastic membrane. Scattered in the intima in the lower part of the picture and deep in the intima close to the internal elastica in the upper part of the picture are small deposits of lipid. Note the marked similarity to the rabbit coronary arteriosclerosis shown in Figure 3 (Weigert-hematoxylin and eosin, × 78).

Fig 5—Atherosclerosis of the right coronary artery of rabbit 10866 (group III) that received semisynthetic lipid-rich diet I and 7 injections of horse serum over a period of 9 months. Average total cholesterol in serum, 157 mg%. Atheromatous intimal thickening with straightening, fragmentation, and reduplication of internal elastic membrane. Fatty-proliferative changes with very little elastic tissue overlie a layer of musculoelastic intimal thickening. Note marked similarity to the human atherosclerosis shown in Figure 6. In the right lower quarter of the picture, the atheromatous intima overlies a thinned media, as commonly occurs in human atherosclerosis. The atheromatous changes seen here did not occur in major coronary arteries of rabbits that received semisynthetic lipid-rich diets *without* injections of foreign serum protein. Figures 13, 15, 17 and 26 are also from this rabbit (Weigert-hematoxylin and eosin, × 96).

Fig 6—Atherosclerosis of the right coronary artery of a 35-year-old man with marked coronary atherosclerosis, old and recent myocardial infarcts, and aortic and mitral stenosis (autopsy 21154, The New York Hospital). Total cholesterol in serum 1 month before death, 279 mg%. Fatty-proliferative changes with pooling of lipid and very little elastic tissue overlie a layer of musculoelastic intimal thickening. Note the very similar changes in the rabbit atherosclerosis shown in Figures 5 and 7. Figure 9 is also from this patient (Weigert-hematoxylin and eosin, × 30).

Fig 7—Atherosclerosis of the left coronary artery of rabbit 10617 (group III) that received semisynthetic lipid-rich diet I and 13 injections of foreign serum protein over a period of 13 months. Average total cholesterol in serum, 311 mg%. Fatty-proliferative changes with pooling of lipid and a small amount of elastic tissue overlie a layer of musculoelastic intimal thickening. Note marked similarity to the human atherosclerosis shown in Figure 6. Figure 11 is also from this rabbit (Weigert-hematoxylin and eosin, × 48).

Fig 8—Atherosclerosis of the left coronary artery of rabbit 10421 (group III) that received semisynthetic diet I and 8 injections of horse serum over a period of 10 months. Average total serum cholesterol, 206 mg%. Fibromuscular intimal thickening with fragmentation of internal elastic membrane and pools of lipid deep in the intima and in the media and with necrosis and marked thinning of the media of the lower segment. Note the marked similarity to the human atherosclerosis shown in Figures 9 and 10. Figure 12 is also from this rabbit (Hematoxylin and eosin, × 64).

Fig 9—Atherosclerosis of the human coronary artery referred to in Figure 6. Fibromuscular intimal thickening with fragmentation and reduplication of the internal elastic membrane and a large pool of lipid deep in the intima. Note the marked resemblance to the rabbit coronary atherosclerosis shown in Figure 8 (Weigert-hematoxylin and eosin, × 20).

Fig 10—Atherosclerosis of the left coronary artery of a 19-year-old man who died with disseminated lupus erythematosus (autopsy 21578, The New York Hospital). Average total cholesterol in serum in last 2 years of life, 220 mg%. Fibromuscular intimal thickening with pooling of lipid deep in the intima. Note the striking similarity to the rabbit atherosclerosis shown in Figures 8, 11 and 12. Figures 16 and 18 are also from this man (Weigert-hematoxylin and eosin, × 40).

Fig 11—Atherosclerosis of the left coronary artery of the rabbit referred to in Figure 7. Fatty-proliferative intimal thickening with pooling of lipid deep in the intima. Note the striking similarity to the human atherosclerosis shown in Figure 10. (Weigert-hematoxylin and eosin, × 80).

Fig 12—Atherosclerosis of the left coronary artery of the rabbit referred to in Figure 8. Fibromuscular and hyaline intimal thickening with large pools of lipid deep in the intima and in the media and with necrosis and thinning of the media. Note the similarity to the human atherosclerosis shown in Figure 10 (Hematoxylin and eosin, × 78).

Fig 13—Atherosclerosis of the rabbit coronary artery referred to in Figures 5, 15, 17 and 26. Fatty-proliferative and hyaline intimal thickening with fragmentation and reduplication of the internal elastic membrane. These changes are strikingly like those of the human atherosclerosis shown in Figure 14 (Weigert-hematoxylin and eosin, × 192).

Fig 14—Atherosclerosis of the left coronary artery of a 46-year-old man with diabetes mellitus and hypertension (autopsy 22653, The New York Hospital). Average total cholesterol in serum in last few years of life, 235 mg%. Fatty-proliferative and hyaline intimal thickening with fragmentation and reduplication of the internal elastic membrane. These changes are strikingly like the rabbit atherosclerosis shown in Figure 13 (Weigert-hematoxylin and eosin, × 120).

Fig 15—Atherosclerosis of the right coronary artery of the rabbit referred to in Figures 5, 13, 17 and 26. Fatty-proliferative and hyaline intimal thickening with fragmentation and reduplication of internal elastic membrane very similar to the human atherosclerosis shown in Figure 16 (Weigert-hematoxylin and eosin, × 192).

Fig 16—Atherosclerosis of the right coronary artery of the man referred to in Figures 10 and 18. Note similarity to the rabbit atherosclerosis shown in Figure 15 (Weigert-hematoxylin and eosin, × 120).

Fig 17—Atherosclerosis of the right coronary artery of the rabbit referred to in Figures 5, 13, 15 and 26. Necrosis of media, fragmentation of internal elastic membrane, and fatty-hyaline intimal thickening. Note the striking similarities to the changes in the human artery shown in Figure 18 (Weigert-hematoxylin and eosin, × 192).

Fig 18—Atherosclerosis of the right coronary artery of the man referred to in Figures 10 and 16. Necrosis of media, fragmentation of internal elastic membrane and fatty-hyaline intimal thickening strikingly like the changes in the rabbit artery shown in Figure 17 (Weigert-hematoxylin and eosin, × 120).

Fig 19—Atherosclerosis of the left coronary artery of rabbit 10701 (group III) that received semisynthetic diet I and 13 injections of horse serum over a period of 16 months. Average total cholesterol in serum, 375 mg%. Proliferative, fatty-proliefrative, and hyaline thickening of the intima with fragmentation of the internal elastic membrane. Note that the greatest intimal *thickening* appears to be associated with the greatest medial *thinning*. The fatty-proliferative intimal thickening in the left upper quarter of the picture and in the right lower quarter may represent lipid deposit in regions of preexisting proliferative intimal change like that in the left lower quarter. Note the cholesterol clefts deep in the intima as in the human artery in Figure 21 (Verhoeff, × 192).

Fig 20—Photograph of a serial section very close to that in Figure 19 to show in higher magnification the cholesterol clefts deep in the intima and close to the fragmented internal elastic membrane, as in the human atherosclerosis shown in Figure 21 (Verfhoeff, × 300).

Fig 21—Atherosclerosis of the right coronary artery of a 57-year-old man who died of congestive heart failure with mitral and aortic valvular disease (autopsy 20235, The New York Hospital). Fatty-proliferative intimal change with cholesterol clefts deep in the intima and close to the fragmented internal elastic membrane, as in the rabbit atherosclerosis shown in Figures 19, 20 and 23 (Weigert-hematoxylin and eosin, × 120).

Fig 22—Atherosclerosis of a major coronary artery of the man referred to in Figure 14. Fatty-hyaline thickening and *vascularization* of the intima with accumulation of lipid at the internal elastic membrane. These changes are strikingly like those of the rabbit atherosclerosis shown in Figure 23 (Weigert-hematoxylin and eosin, × 120).

Fig 23—Atherosclerosis of the right coronary artery of rabbit 10407 (group III) that received semisynthetic, lipid-rich diet I and 8 injections of bovine serum albumin over a period of 12 months. Average total cholesterol in serum, 285 mg%. Fatty-hyaline thickening and *vascularization* of the intima with accumulation of lipid and cholesterol clefts close to the internal elastic membrane. These changes are strikingly like those of the human atherosclerosis shown in Figures 21 and 22 (Weigert-hematoxylin and eosin, × 192).

Fig 24—Atherosclerosis of mesenteric artery of rabbit 10406 (group III) that received semisynthetic, lipid-rich diet I and 6 injections of bovine serum albumin over a period of 1 year. Average total cholesterol in serum, 280 mg%. Fatty-hyaline intimal thickening with fibromuscular cap and deep intimal deposits of calcium is associated with foam cells. Note the striking similarity to the human atherosclerosis shown in Figure 25 (H & E, × 192).

Fig 25—Atherosclerosis of the left coronary artery of a 72-year-old man who died with disseminated rheumatoid disease (autopsy 23238, The New York Hospital). Serum cholesterol content not known. Fatty-hyaline intimal thickening with fibromuscular cap and deep intimal deposits of calcium is associated with clusters of foam cells. Note the striking similarity to the rabbit atherosclerosis shown in Figure 24 (H & E, × 120).

Fig 26—Atherosclerosis with organizing thrombosis of the left coronary artery of the rabbit referred to in Figures 5, 13, 15 and 17 (H & E, × 102).

Fig 27—Sulcus of the mitral valve of rabbit 10629 (group III) that received semisynthetic, lipid-rich diet I and 6 injections of horse serum over a period of 6 months. Average total cholesterol in serum, 184 mg%. Fatty-hyaline thickening of the base of the valve with overlying thrombus (H & E, × 80).

Fig 28—Sulcus of the aortic valve of rabbit 10727 (group III) that received semisynthetic, lipid-rich diet I and 10 injections of foreign serum protein over a period of 12 months. Average total cholesterol in serum, 158 mg%. Fatty-hyaline thickening of the base of the valve with stigmata of thrombosis deep in the lesion at lower right (H & E, × 50).

Fig 29—Fatty virtually acellular change in the intima of a small intramyocardial artery in rabbit 10928 (group I) that received semisynthetic, lipid-rich diet II over a period of 8 months without injections of foreign protein. Average total cholesterol in serum, 170 mg%. This was the characteristic arterial lesion in the hearts of animals of group I (H & E, × 320).

Fig 30—Proliferative intimal thickening of small intramyocardial artery of the rabbit referred to in Figure 29. Identical small artery changes were found to occur spontaneously in many rabbits not in these experiments (H & E, × 320).

Fig 31—Arch of aorta of rabbit 10640 (group I) that was fed semisynthetic, lipid-rich diet I for 21 months without injections of horse serum. Average total cholesterol in serum, 260 mg%. Fatty change in intima and media (H & E, × 120).

Fig 32—Ascending aorta of rabbit 11577 (group II) that received the lipid-poor commercial rabbit ration and 10 injections of horse serum at intervals of 2 to 8 weeks. Sacrificed 2 weeks after last injection. Total cholesterol in serum within normal range. Unusually marked degenerative and proliferative changes in intima and media (H & E, × 120).

Fig 33—Arch of aorta of rabbit 10736 (group III) that received semisynthetic, lipid-rich diet I and 9 injections of horse serum over a period of 10 months. Died 1 day after last injection. Average cholesterol in serum, 266 mg%. Degenerative and fatty-proliferative intimal and medial changes very similar to those of the human aortic atherosclerosis shown in Figure 34 (H & E, × 120).

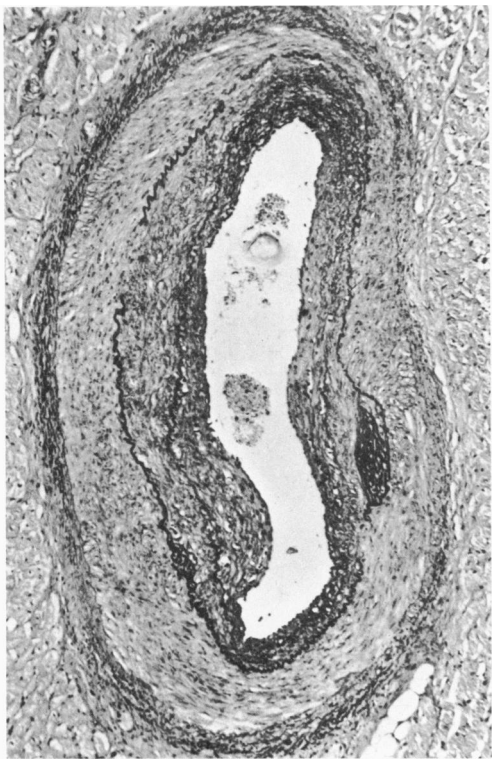
Fig 34—Aortic atherosclerosis in a 24-year-old man who died of congestive heart failure with mitral stenosis and insufficiency and aortic insufficiency (autopsy 11686, The New York Hospital). Serum cholesterol content not known. Degenerative and fatty-proliferative intimal and medial changes very similar to those of the rabbit aortic atherosclerosis shown in Figure 33 (H & E, × 120).

Fig 35—Abdominal aorta of rabbit 10740 (group III) that received semisynthetic, lipid-rich diet I and 12 injections of foreign serum protein over a period of 15 months. Average total cholesterol in serum, 322 mg%. Atherosclerosis with fatty-proliferative and fatty-hyaline intimal and medial changes. Deep in the arterial wall and involving the media are deposits of relatively acellular lipid with cholesterol clefts. Note the marked similarity to the human aortic atherosclerosis shown in Figure 36 (H & E, × 78).

Fig 36—Aortic atherosclerosis in a 46-year-old man who died of dissecting aneurysm of the aorta with arteriosclerotic, hypertensive and active rheumatic heart disease (autopsy 18257, The New York Hospital). Serum cholesterol content not known. Fatty-proliferative and fatty-hyaline intimal and medial changes, with lipid deposit deep in the wall and involving the media. Note the marked similarity to the rabbit aortic atherosclerosis shown in Figure 35 (H & E, × 78).

Fig 37—Arch of aorta of the rabbit referred to in Figure 35. Atherosclerosis with fatty-proliferative and fatty-hyaline intimal and medial changes. The deepest deposits of lipid, including cholesterol clefts, involve the media. Note the close resemblance of this rabbit aortic atherosclerosis to the human aortic atherosclerosis shown in Figure 38 (H & E, × 75).

Fig 38—Aortic atherosclerosis in a 62-year-old man who received a cardiac homograft 4 months before death because of occlusive coronary atherosclerosis (autopsy 24362, The New York Hospital). Total cholesterol in serum a few weeks before death, 221 mg%. Fatty-proliferative and fatty-hyaline intimal and medial changes. The deepest deposits of lipid involve the media. Note the close resemblance of this human aortic atherosclerosis to the rabbit aortic atherosclerosis shown in Figure 37 (H & E, × 60).



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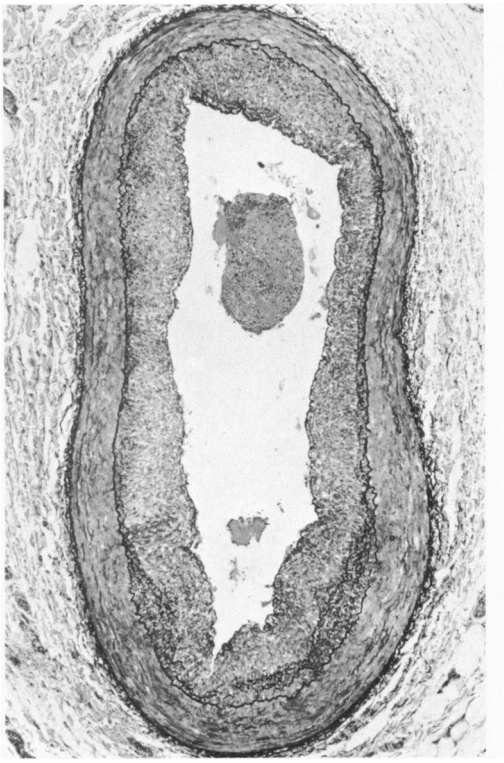

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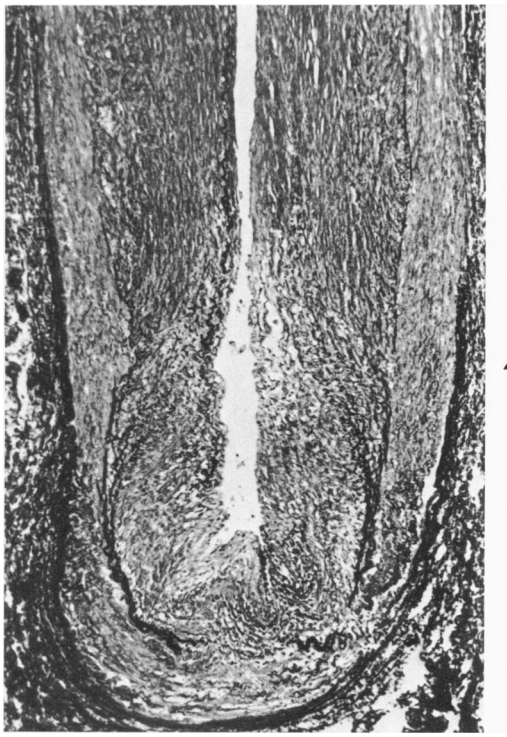

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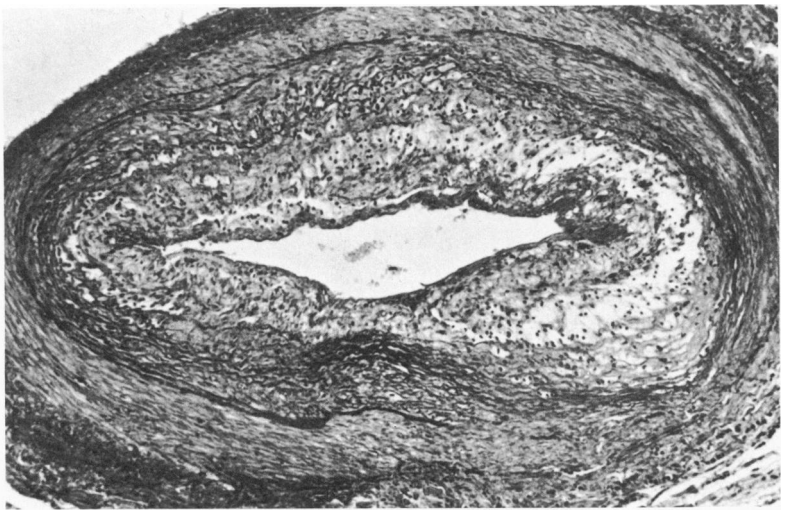

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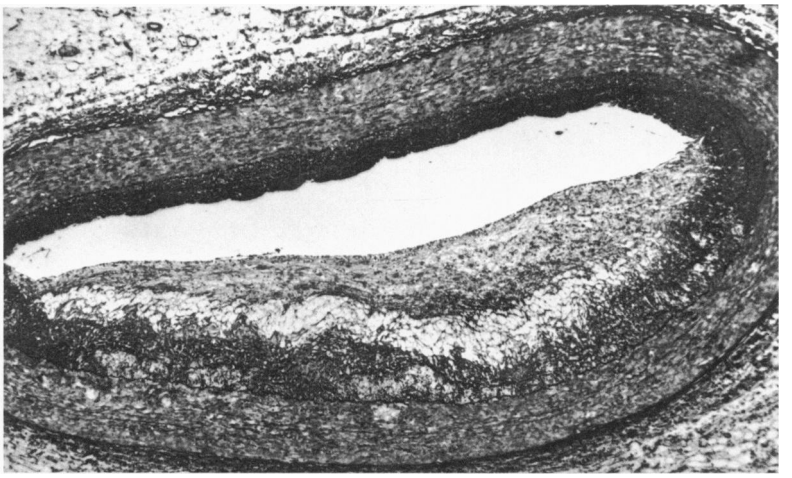

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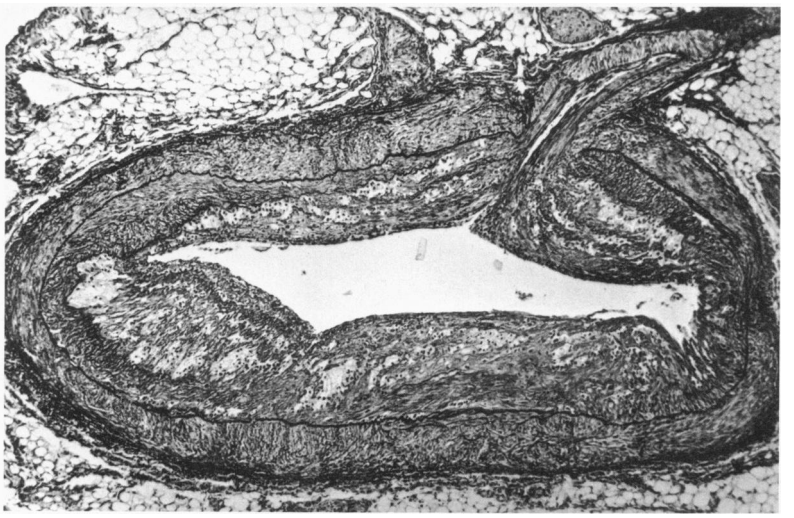

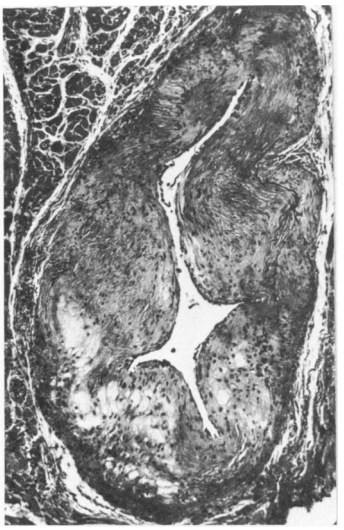

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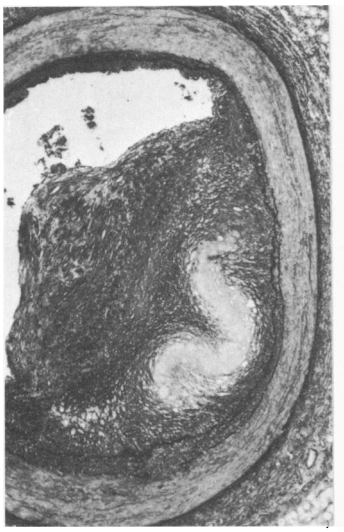

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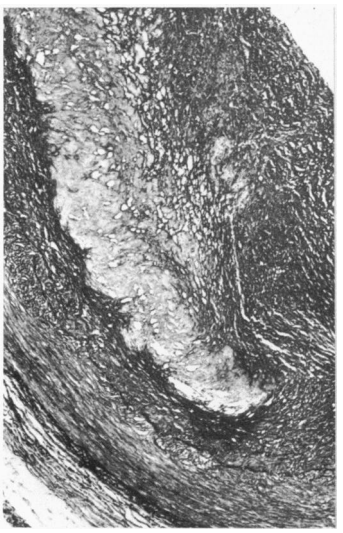

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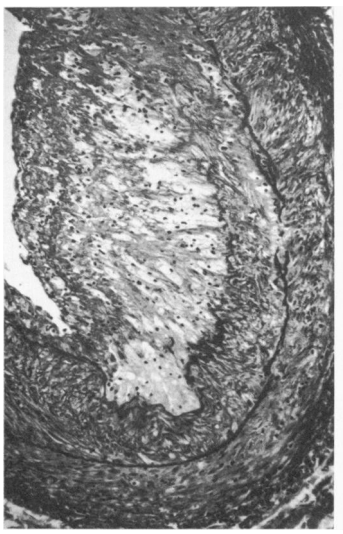

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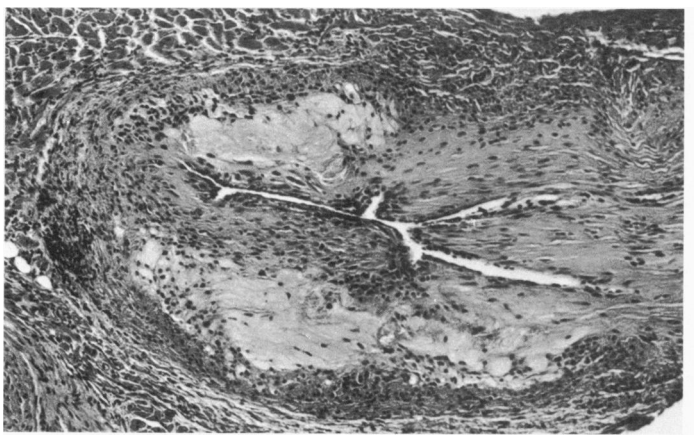

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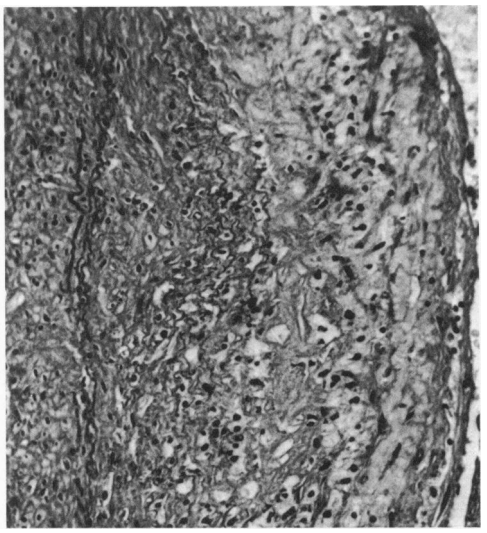

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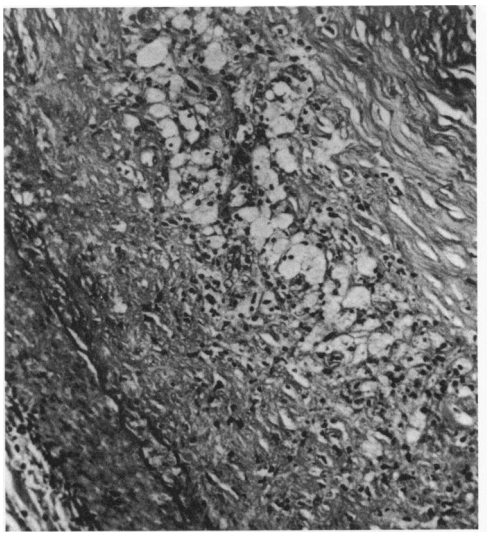

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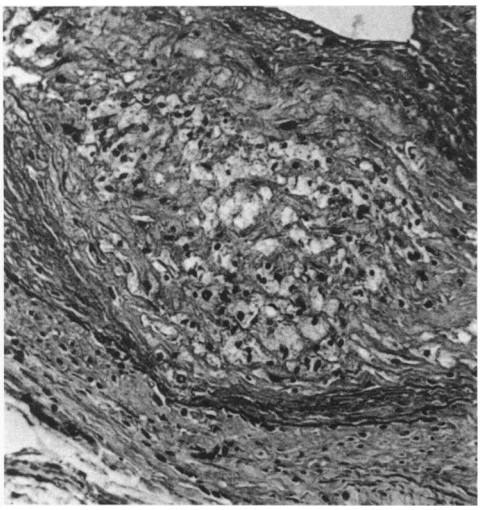

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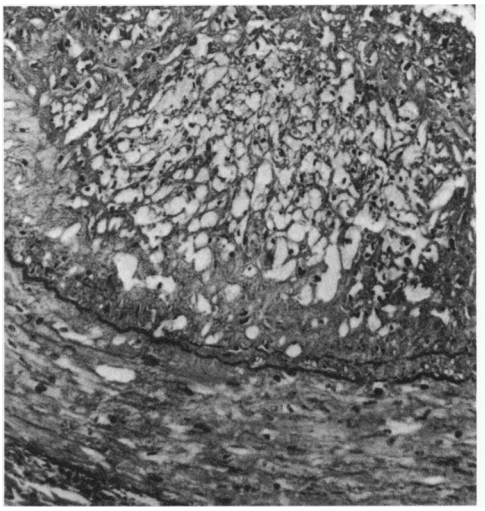

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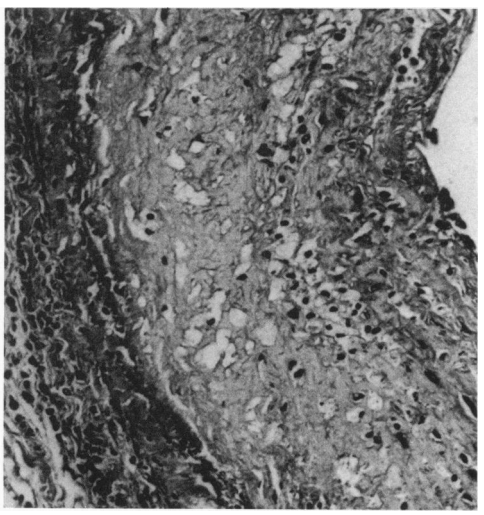

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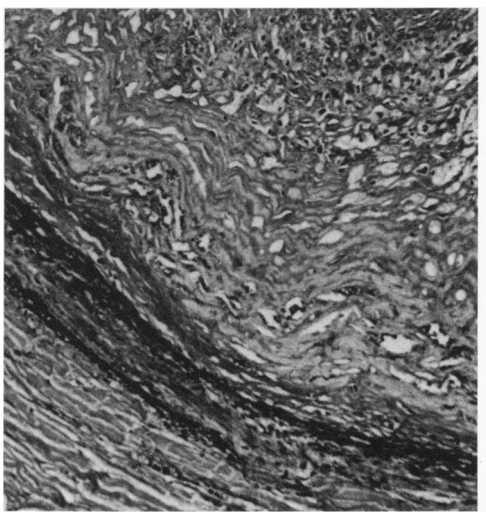

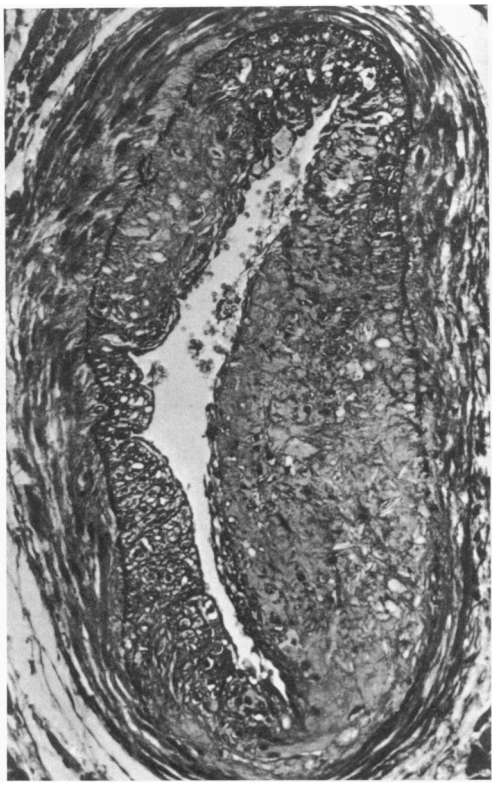

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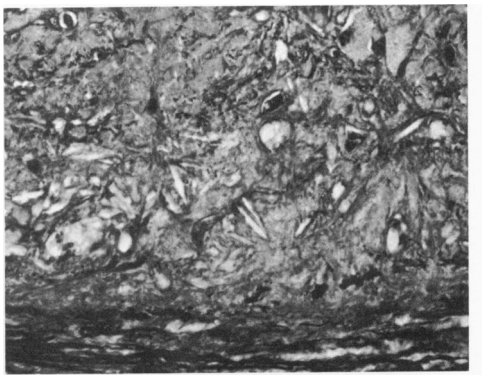

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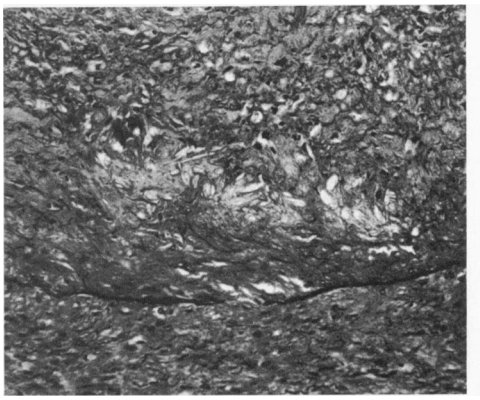

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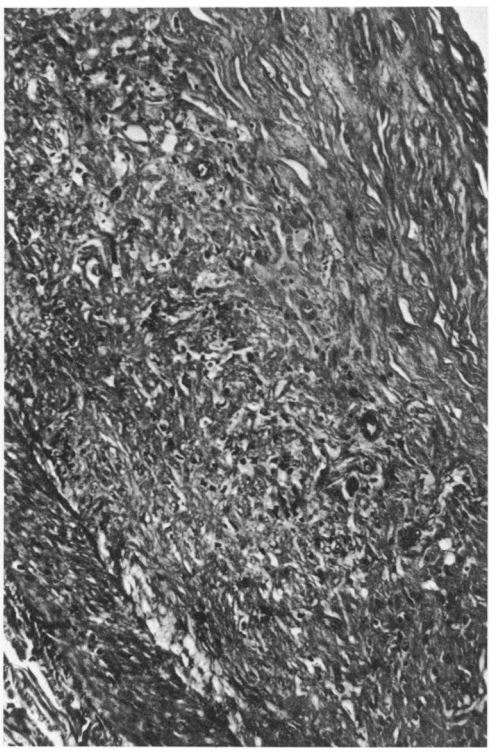

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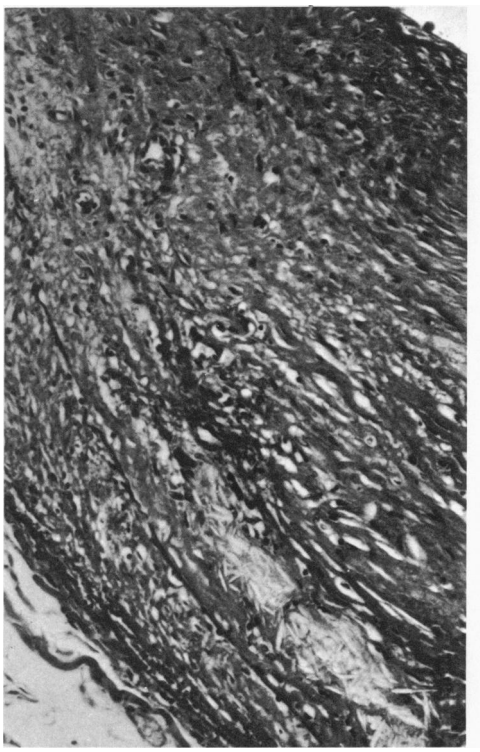

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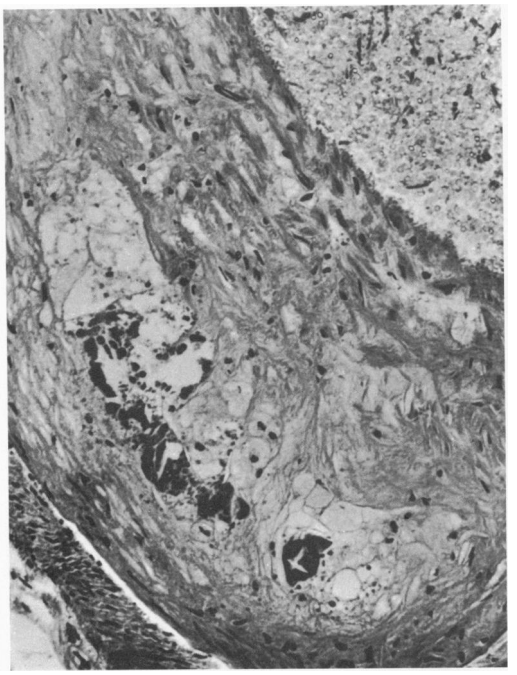

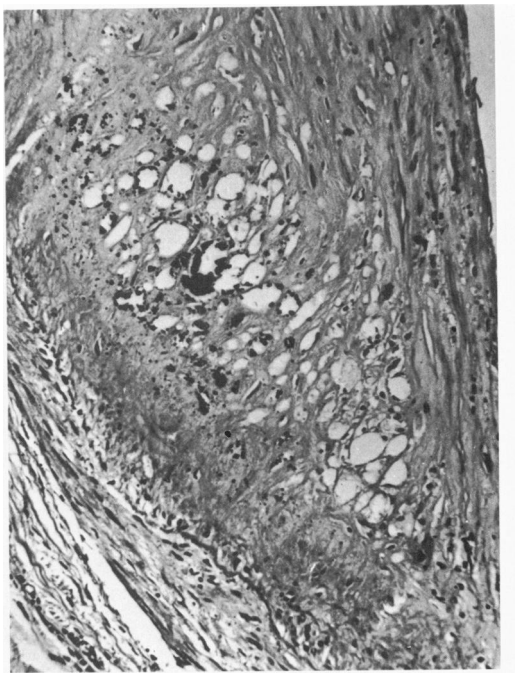

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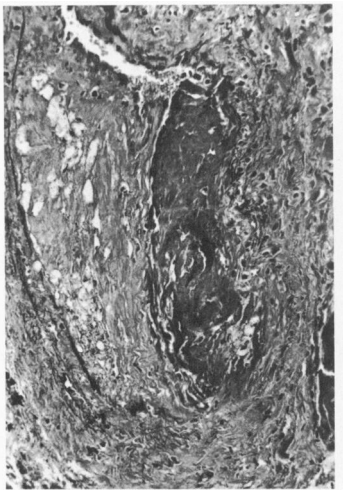

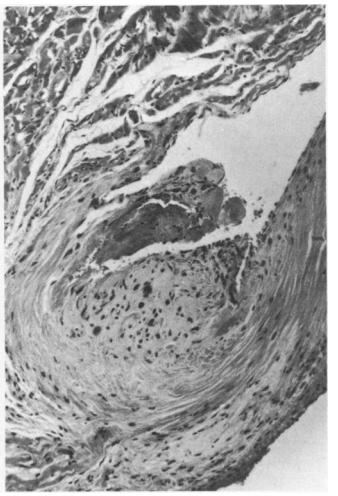

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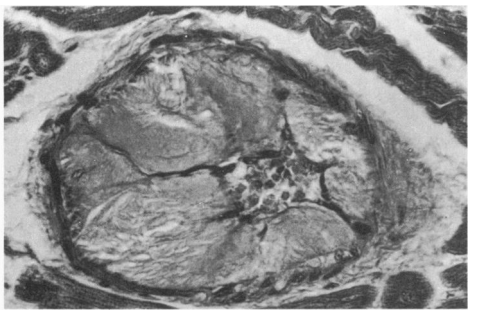

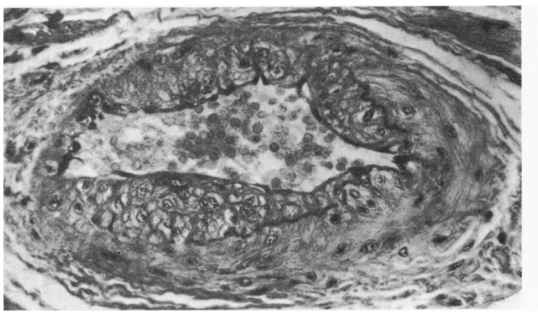

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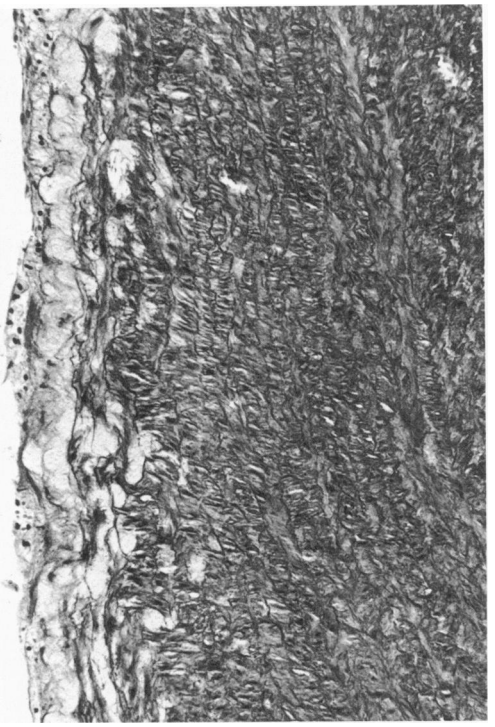

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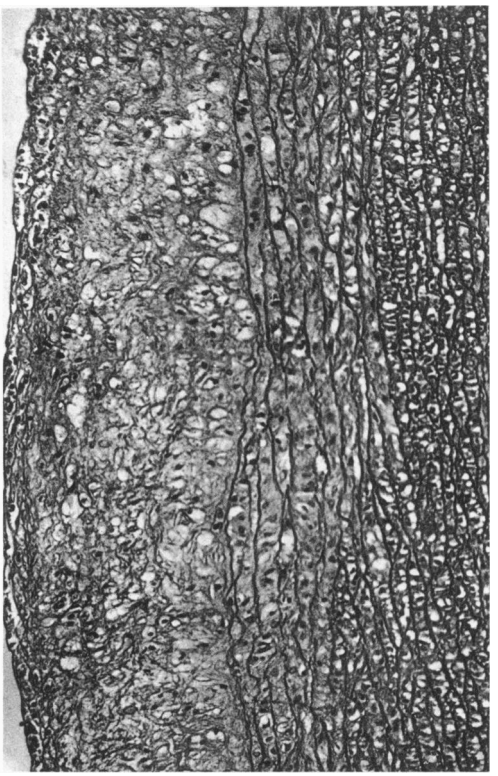

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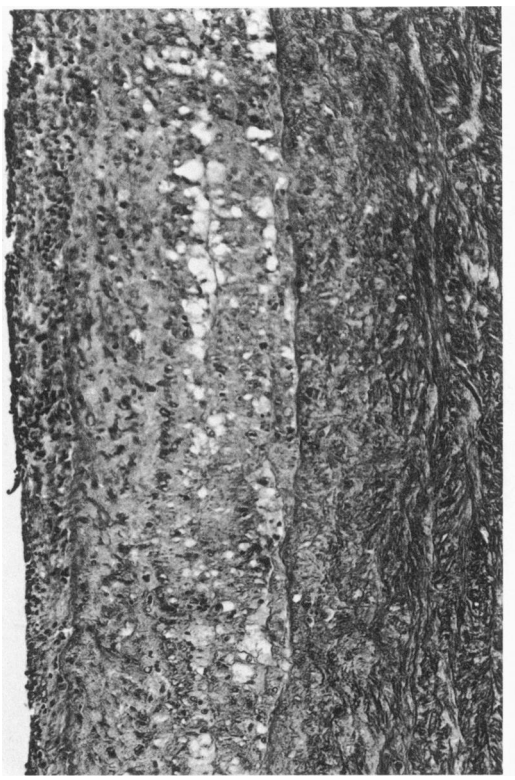

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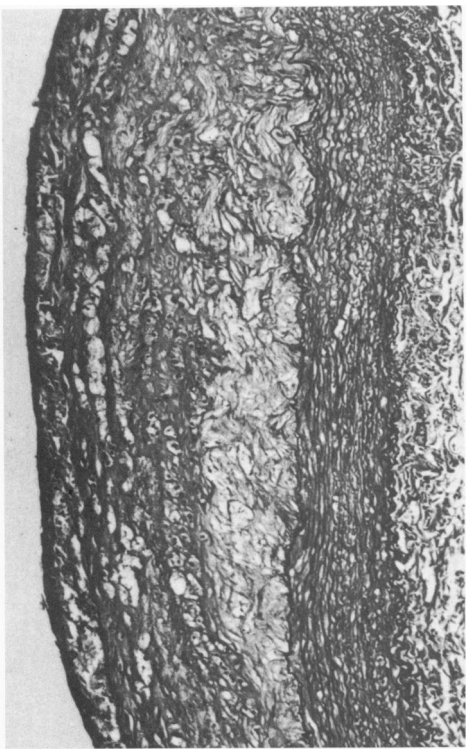

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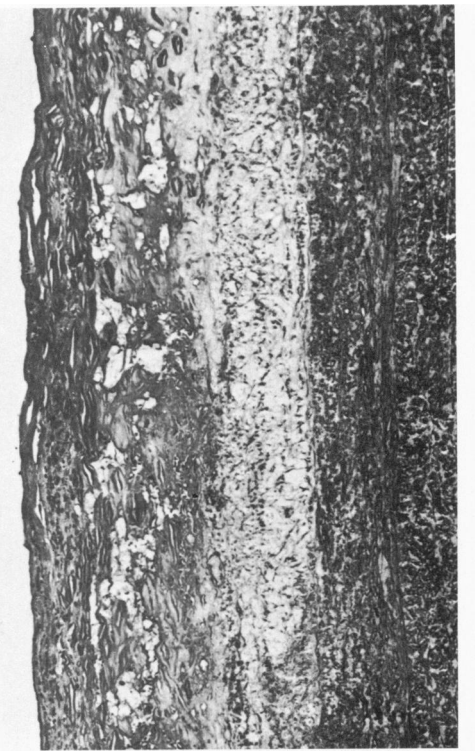

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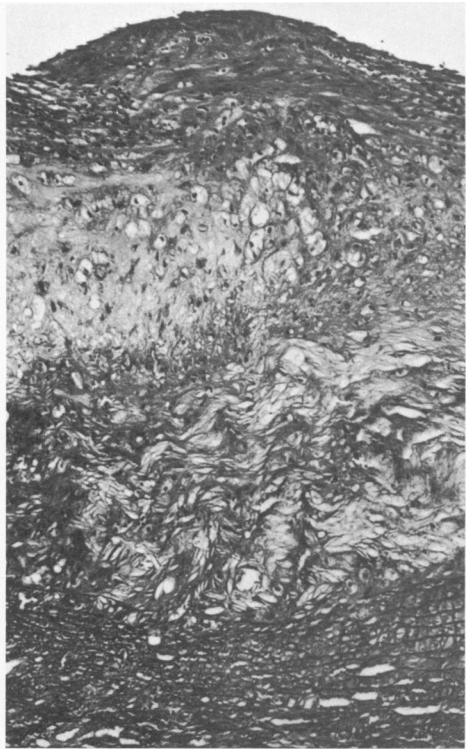

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