EXPERIMENTAL INDUCTION OF ATHERO-ARTERIOSCLEROSIS
BY THE SYNERGY OF ALLERGIC INJURY TO ARTERIES
AND LIPID-RICH DIET

I. EFFECT OF REPEATED INJECTIONS OF HORSE SERUM IN RABBITS FED A DIETARY CHOLESTEROL SUPPLEMENT

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Injury to the arterial wall is probably a primary causative factor in arterial atherosclerosis (athero-arteriosclerosis) (1–6). The injury and local changes that develop in reaction to it may favor deposition and accumulation of blood-borne lipid at the site of the reaction. To discover the causes of the injury and determine the nature of the local reactive changes are of basic importance to the understanding of human athero-arteriosclerosis. In this connection, the likelihood that syphilitic injury to the aorta can lead to aortic atherosclerosis comes to mind (1, 7, 8). In a large proportion of attacks of rheumatic fever, injury to cardiac valves occurs and can lead to valvular sclerosis, and in some cases atherosclerosis evolves. Rheumatic injury to small and medium sized arteries, especially of the heart, also occurs frequently and can lead to sclerosis of these vessels (9–16). Furthermore, rheumatic injury to large coronary arteries and the aorta, especially its thoracic portion, can occur and lead to sclerosis of these vessels (13–19). In some of these large arteries, as in some of the cardiac valves, atherosclerosis may evolve (20–22). Data obtained from clinical observation and from experiment indicate that allergy is probably a very important causative factor in rheumatic cardiovascular disease (16, 23–27). Allergy may also be an important causative factor in syphilitic cardiovascular disease (28, 29). Allergy to other antigens, including those in infecting micro-

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organisms and vaccines, foreign serum, antibiotics and other drugs, and food, and perhaps those derived from the individual's own tissues, may be a causative factor in an even greater amount of arterial disease (30), some of which may evolve as athero-arteriosclerosis.

The possibility that allergic injury to arteries is a causative factor in some cases of athero-arteriosclerosis is little considered or supported by experimentally acquired data (31, 32). In contrast, the concept that dietary lipid is a causative factor in athero-arteriosclerosis is widely held and is supported by data acquired from experiment (1, 33–36).

The purpose of this communication is to report on testing the hypothesis that the synergy of allergic injury to arteries and lipid-rich diet can lead to athero-arteriosclerosis.

Materials and Methods

Eighty young rabbits, 75 gray and 5 New Zealand red, weighing from 1500 to 3000 g, were fed Rockland rabbit pellets, of which the total lipid does not exceed 1.5% by weight. In addition, many were fed a supplement of cholesterol (Cholesterin, U.S.P., Merck and Co., Inc., Rahway, New Jersey). The cholesterol was dissolved in ether, and Rockland rabbit pellets were coated with the solution. The ether was then evaporated. The amount of cholesterol in this diet was 0.5% by weight. All rabbits were given water ad libitum. Unpreserved horse serum, supplied by Lederle Laboratories, Pearl River, New York, was passed through a Seitz filter just before use. In sacrifice of animals veterinary sodium nembutal was given intravenously.

The rabbits were divided into three groups as follows:

Group I comprised 25 rabbits. They were fed 100 g of cholesterol-coated pellets 5 days per week for 74 to 80 days. Fifteen (group I-A) were sacrificed between days 80 and 90 of the experiment, or within 10 days after cessation of feeding cholesterol. The remaining 10 (group I-B) were sacrificed between days 140 and 150 of the experiment, or between 60 and 70 days after cessation of feeding cholesterol.

Group II comprised 17 rabbits. They were fed 100 g of Rockland rabbit pellets without cholesterol supplement. In addition, they received intravenously 4 injections, spaced 16 to 18 days apart, of 10 cc/kg of sterile horse serum. 18 to 56 hr before each of the last three injections a desensitizing dose of 1 cc of horse serum was given intravenously. Fourteen (group II-A) were sacrificed within a week after and the remaining 3 (group II-B) between 60 and 75 days after the last large injection of horse serum.

Group III comprised 38 animals. They were fed 100 g of cholesterol-coated pellets 5 days per week for 79 to 84 days. Beginning 22 to 24 days after institution of cholesterol feeding they received injections of horse serum as outlined in the preceding paragraph. Twenty-four (group III-A) were sacrificed within a week after the fourth large injection. Another of this group died one day after the desensitizing injection prior to the scheduled fourth large injection. The remaining 13 (Group III-B) were sacrificed 60 to 75 days after the last large injection of horse serum.

Approximately half of the animals in groups I, II, and III were bled from the marginal ear vein every 10 to 20 days. Total serum cholesterol was estimated by the method of Abel, Levy, Brodie, and Kendall (37). Total serum lipid values were approximated by a gravimetric procedure of Abel and Kendall (38). 1 ml of serum and 15 ml of absolute ethyl alcohol in a 25 ml volumetric flask were shaken, heated to boiling in a water bath, and cooled. 7 ml of diethyl
ether were added and the mixture brought to 25 ml with absolute ethyl alcohol was shaken and filtered. 15 ml of filtrate were placed in a glass tube and evaporation carried to dryness. To the residue 0.1 ml of 0.5 N HCl in 50% ethanol and then 15 ml of petroleum ether were added and the tube vigorously shaken. After clearing, 10 ml of this petroleum ether extract were placed in a previously weighed bottle, evaporation carried to dryness, and the weight of the residue determined.

Autopsies were performed on all animals. The hearts and aortas were removed and fixed in buffered formalin. Multiple small blocks of tissue were removed from the lungs, liver, gall bladder, spleen, pancreas, skeletal muscle, stomach, small intestine, large intestine, kidneys, adrenals, and mesentery of the small intestine, and brain. Some blocks were fixed in Zenker's acetic acid and others in buffered formalin.

The heart was dissected to obtain one or more blocks of the following: (1) right ventricular wall, proximal portion of right main coronary artery, interventricular septum, right coronary cusp of aortic valve, and base of aorta; (2) left ventricular wall, anterior papillary muscle, and base of mitral valve; (3) left ventricular wall, posterior papillary muscle, base of mitral valve, and adjacent atrial wall; and (4) left ventricular wall, left coronary cusp of aortic valve, and base of aorta. Blocks 2 to 4 often included the left main coronary artery and/or its major branches. Blocks of the hearts, thoracic aortas, and other organs were embedded in paraffin and sections cut at 5 μ were stained routinely with hematoxylin and eosin. Many sections with cardiovascular lesions were stained with either Weigert-van Gieson, Weigert-hematoxylin and eosin, or Verhoeff's elastic stain, or by Masson trichrome method. Formalin-fixed blocks of the heart and other tissues from some rabbits were embedded in a warmed solution containing 7.5 g of gelatin in 100 cc of 10% formalin. These embedded blocks were frozen and sections cut at 5 μ were stained with oil red O (a Sudan stain) and hematoxylin. These blocks were subsequently embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

For purposes of microscopic analysis coronary arteries were defined as large, medium, or small as follows: main arteries and major branches were classified as large; intramyocardial arteries that were not major branches but more than filled a microscopic field when a Zeiss 40X objective lens and Zeiss Kpl. 12.5X ocular lenses were used, were classified as medium; and all other intramyocardial arteries were classified as small.

The aortas of all rabbits were opened in entirety and examined. Photographs of the luminal aspects of 53 randomly sampled aortas were made on 35 mm Kodachrome, type F film. In two of them spontaneously occurring degenerative changes were present and these were excluded from the analysis to be described. Of the remaining sample of 51 aortas, 16 were from rabbits that received the cholesterol supplement without horse serum (group I), 14 from rabbits that received horse serum without the cholesterol supplement (group II), and 21 from rabbits that received the cholesterol supplement and horse serum (group III). The Kodachrome transparencies were placed on an X-ray illuminating box and, one with the other, first the thoracic portions were compared and then the abdominal portions were compared without reference to the treatment the animals received. According to the amount of change observed and relative to one another, first each thoracic and then each abdominal portion of aorta was placed in one of the following groups: Those with marked change, those with intermediate change, those with slight change, and those with no change observable.

For the purpose of morphologic comparison between experimentally induced coronary athero-arteriosclerosis in rabbits and naturally occurring coronary athero-arteriosclerosis in man and between the former and experimentally induced coronary arteriosclerosis without lipid change, photographs of coronary arteries of rabbits of group III are submitted alongside those of two humans (Figs. 3, 7, 8, and 12) and two rabbits (Figs. 1, 15, 16, 21, and 23). The latter rabbits, like those in group II, received Rockland rabbit pellets without cholesterol supplement and concomitant injections of horse serum. One of these rabbits received 2 large
injections of horse serum spaced 17 days apart and the other 6 large injections of horse serum and 6 large injections of swine serum over a period of 6 months. One of the humans was a 35-yr-old man who died of advanced rheumatic heart disease with marked calcific stenosis and insufficiency of the mitral and aortic valves, marked athero-arteriosclerosis of the right and left coronary arteries, and old and recent infarcts of the interventricular septum and lateral wall of the left ventricle. The total cholesterol content in his serum was 279 mg/100 ml and his blood pressure was 145/50 1 month before death. The other was a 19-yr-old man who died of chronic proliferative and membranous glomerulonephritis established at autopsy. Pronounced athero-arteriosclerosis of coronary arteries, especially the right, was also present. Since childhood he had experienced repeated attacks of asthma and many episodes of urticaria, some of which followed the ingestion of aspirin. 22 months before death there was acute onset of renal disease with proteinuria, hematuria, and edema. 3 wk later the serum antistreptolysin O titer was less than 25 Todd units and 1 month later 25 Todd units. 10 days later complement-fixing antibodies to calf thymus nuclei in a titer of 1 to 16 and to calf thymus DNA in a titer of 1 to 4 were present in the patient's serum. In a Hyland slide agglutination test latex particles coated with calf thymus nucleoprotein were agglutinated (2+, in a scale of 0 to 4+) in reaction with the patient's serum. A specimen of the right kidney was obtained in open biopsy. In addition to the histologic features of marked diffusely proliferative glomerulonephritis there were found in glomeruli many "hyaline thrombi" in capillary lumina and foci of hypereosinophilic thickened capillary walls with "wire-loop" appearance like those that frequently occur in disseminated lupus erythematosus. A few days later a lupus erythematosus cell test of the patient's blood was positive. The total cholesterol in his serum ranged from 192 to 259 mg/100 ml in 6 determinations during the last 18 months of life, and his blood pressure remained normal until hypertension developed 3 months before death.

RESULTS

**Serum Lipids.**—In groups I and III, as recorded in Text-fig. 1, the estimated average total cholesterol and average total lipid in serum increased similarly during the period of cholesterol feeding and reached peak level in the 2 wk before sacrifice as follows: cholesterol in group I from an initial value of 42 mg/100 ml to between 650 and 677 mg/100 ml, and in group III from an initial value of 52 mg/100 ml to between 636 and 721 mg/100 ml; total lipid in group I from an initial value of 477 mg/100 ml to between 1357 and 1449 mg/100 ml and in group III from an initial value of 361 mg/100 ml to between 1149 and 1373 mg/100 ml. In those animals in groups I and III that were sacrificed 60 to 75 days after cessation of cholesterol feeding, the average total cholesterol had decreased to 277 and 236 mg/100 ml respectively, and the average total lipid had decreased to 636 and 591 mg/100 ml, respectively, prior to sacrifice. In group II there was no increase in the average total cholesterol or average total lipid in serum in the course of the experiment. The average total cholesterol varied from 48 to 134 mg/100 ml and the average total lipid from 212 to 528 mg/100 ml.

**Arterial lesions** developed in rabbits of all groups. Their distribution and

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1 These determinations were made by Dr. William C. Robbins in a semiquantitative test in which a modification of a previously reported technique (39) was used.
qualitative characteristics varied with treatment. Of the 25 animals in group I lesions were found in the pulmonary arteries of the majority and aortas of all. Arterial lesions were also found in hearts of 18, lungs of 5, and kidneys of 1. Of the 17 animals in group II lesions were found in the main pulmonary arteries of several and ascending aortas of many. Arterial lesions were also found in hearts of 15, lungs of 11, mesenteries of 5, splenic arteries of 4, and kidneys of 3. Of the 38 animals in group III lesions were found in the main pulmonary arteries of the majority and aortas of all. Arterial lesions occurred in hearts of 28, lungs of 30, kidneys of 16, mesenteries of 12, and splenic arteries of 4. In group I the arterial changes were fatty lesions and, except for those in the aortas and pulmonary arteries, occurred almost solely in small, rarely in medium arteries. In group II the arterial changes were proliferative lesions without fatty change and the frequency of lesions in large, medium, and small arteries was approximately the same. In group III the arterial changes were of three types: fatty, proliferative, and fatty-proliferative. Except in the aortas and main pulmonary arteries where the lesions were fatty, the large majority of arterial changes were fatty-proliferative lesions. The frequency of lesions in large, medium, and small arteries was approximately the same. The changes in the coronary arteries and aortas are reported in detail.

Text-Fig. 1. Average total cholesterol in sera of rabbits of groups I and III in the course of and following the feeding of a diet containing a cholesterol supplement of 0.5% by weight.
Coronary Arterial Lesions.—In group I, as shown in Table I, arterial changes were confined almost solely to small arteries, 96% of them occurring in vessels of this calibre, and were never observed in large arteries. Intimal cells and medial smooth muscle cells were distended with lipid (Fig. 27) which stained orange-red with oil red O. No evidence of regression of or appreciable proliferative change in these fatty lesions was provided by comparison of the lesions of small myocardial arteries in rabbits sacrificed 10 days after with those in rabbits sacrificed 60 to 70 days after cessation of the feeding of the cholesterol supplement.

In group II changes were present throughout the coronary arterial tree. 22% of the lesions observed were in large arteries, 36% in medium arteries, and 42% in small arteries. These changes were proliferative lesions without fatty change. They were segmental and comprised proliferation of intimal cells, musculo-elastic hyperplastic intimal change, focal fragmentation and/or reduplication of internal elastic membrane, degenerative and proliferative changes in the media, focal thinning and scarring of media, and cellular proliferative and infiltrative change and fibrosis in adventitia. These changes are illustrated in Figs. 1, 21, 23, and 26. Sometimes proliferating cells in the intima contained nuclei with caterpillarlike chromatin pattern or owl eye appearance like those in some reacting medial smooth muscle cells (Figs. 15, 16, and 23). In arteries of animals sacrificed 60 to 75 days after the last injection of foreign serum there

<table>
<thead>
<tr>
<th>Regimen</th>
<th>No. of animals that developed arterial lesions (% of group)</th>
<th>No. of arterial lesions examined</th>
<th>Distribution of arterial lesions (% of lesions)</th>
<th>Incidence of histologic types of arterial lesions</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Large arteries Medium arteries Small arteries</td>
<td>Fatty proliferative Fatty proliferative</td>
</tr>
<tr>
<td>Group I</td>
<td>Fed cholesterol supplement (25 rabbits)</td>
<td>19 (76%)</td>
<td>55</td>
<td>0 2 53 (4%) (96%) 100% 0 0</td>
</tr>
<tr>
<td></td>
<td>Injected repeatedly with horse serum (17 rabbits)</td>
<td>15 (88%)</td>
<td>90</td>
<td>20 32 38 (22%) (36%) (42%) 0 100% 0</td>
</tr>
<tr>
<td>Group II</td>
<td>Fed cholesterol supplement and injected repeatedly with horse serum (38 rabbits)</td>
<td>29 (76%)</td>
<td>97</td>
<td>28 32 37 (29%) (33%) (38%) 4% 6% 90%</td>
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TABLE I

Incidence, Distribution, and Histologic Types of Coronary Arterial Lesions
was sometimes slight to moderate proliferation of intimal cells and medial smooth muscle cells in addition to advanced muscular and/or elastic hyperplastic intimal changes (Figs. 15, 16, 21, and 23).

In group III changes were present throughout the coronary arterial tree. 29% of the lesions observed were in large arteries, 33% in medium arteries, and 38% in small arteries. These changes were of three types: fatty, proliferative, and fatty-proliferative. The great majority were fatty-proliferative and they accounted for 90% of the arterial lesions. The characteristics of fatty lesions and proliferative lesions are described above. The fatty-proliferative changes were segmental and comprised proliferation of intimal cells, presence of lipid in intimal and medial cells, fatty-hyaline intimal change with lack of or little elastification, focal fragmentation and reduplication of the internal elastic membrane, focal thinning and scarring of media, and cellular proliferative and infiltrative change and fibrosis in adventitia. These changes are illustrated in Figs. 2, 4 to 6, 9 to 11, 13, 14, 17 to 20, 22, 24, and 25. Segmental fatty-proliferative intimal change often overlay fragmented or reduplicated internal elastic membrane and altered, sometimes lipid-laden medial smooth muscle cells (Figs. 2, 4 to 6, 9, 10, 13, 14, 22, 24, and 25). Sometimes intimal and medial cells, including “foam” cells, contained nuclei with caterpillar-like chromatin pattern or owl eye appearance (Figs. 14 and 17 to 20). As compared with many of the advanced proliferative intimal lesions in animals in group II, advanced fatty-proliferative intimal lesions in animals in group III contained only a small amount of elastic fibers, sometimes virtually none, and some exhibited a kind of fatty-hyaline change (Figs. 9, 10, 13, 17 to 19, 22 and 24). In arteries of animals sacrificed 60 to 75 days after the final injection of horse serum there was sometimes in addition to advanced changes slight to moderate proliferation of intimal and medial cells, some of which contained caterpillar or owl eye nuclei and/or lipid (Figs. 17 to 20). Proliferating cells in intima sometimes appeared to form a jacket or cap over or around clusters of “foam” cells (Figs. 5, 6, 13, 17, 22, and 24).

Aortic Lesions.—In the gross, no changes were seen in the lining of aortas of animals in group II. Microscopically, in the ascending portion of many of these aortas foci of proliferated intimal cells were observed that in some cases were associated with fragmentation of subjacent elastic fibers and degenerative change in underlying smooth muscle. These changes were most marked in the first few centimeters of the aorta, including the coronary ostia, and often involved the aortic sulcus.

In the lining of the aortas of all rabbits fed the cholesterol supplement (groups I and III), yellow-white fatty streaks and plaques developed. The thoracic aorta was most involved. On gross examination there was a greater amount of aortic fatty change in the group of rabbits that received the dietary cholesterol supplement and concomitant injections of foreign serum (group III) than in the
group that received the cholesterol supplement but no foreign serum (group I). The greatest difference was in the thoracic aortas and this is shown in Text-fig. 2. Of the 16 examined thoracic aortas of group I, a marked amount of fatty change was present in 4 or 25%, an intermediate amount in 5 or 31%, and a slight amount in 7 or 44%. In contrast, of the 21 examined thoracic aortas of group III, a marked amount of fatty change was present in 15 or 71%, an intermediate amount in 5 or 24%, and a slight amount in 1 or 5%. Of the 16 examined abdominal aortas of group I a marked amount of fatty change was present in none, an intermediate amount in 2 or 12.5%, a slight amount in 12 or 75%, and no change was observed in 2 or 12.5%. Of 20 examined abdominal aortas of group III a marked amount of fatty change was present in 4 or 20%, an intermediate amount in 5 or 25%, and a slight amount in 11 or 55%.

The difference between the amount of fatty change in the thoracic aortas of group I and that of group III is statistically significant as determined by the χ² test (0.01 > P > 0.005). The difference between the amount of fatty change in the abdominal aortas of group I and that of group III is not statistically significant (0.100 > P > 0.050).

Microscopically, the aortic lesions in groups I and III were predominantly
fatty. They comprised lipid-laden intimal cells and subjacent medial smooth muscle cells (Fig. 9). In some animals of both groups there was fatty change in the aortic sulcus and cusps of the aortic valve. The fatty change in the aortas invariably terminated abruptly in the mouths of the coronary arteries. This termination together with the fatty change in an aorta and contrasting fatty-proliferative change in a main coronary artery beyond are illustrated in Figs. 9 and 10. Prominent reparative change like that which occurred in the proliferative and fatty-proliferative arterial lesions did not occur in either the fatty lesions of the aortas or those of small arteries.

**DISCUSSION**

In the experiments here reported rabbits that received a dietary supplement of cholesterol and repeated injections of horse serum (group III) developed arterial changes that in the main were different in quality and distribution from those in rabbits that received the cholesterol supplement alone (group I), and of quality different from but in distribution similar to those in rabbits that received foreign serum alone (group II). The average values of total cholesterol and total lipid in sera of group III were virtually the same as those in group I and much greater than those in group II. In all three of the groups changes occurred in the aorta and in many other arteries, especially of the heart. The lesions in arteries of the heart will be discussed first.

In group I fatty change occurred in intimal and medial cells almost solely of small, rarely of medium, and never of large arteries. In group II proliferative lesions without fatty change developed. They were segmental and comprised proliferation of intimal and medial cells, focal fragmentation and/or reduplication of internal elastic membrane, musculo-elastic hyperplastic intimal change, focal degenerative changes in media, and proliferation and infiltration of cells and fibrosis in adventitia. In contrast with the arterial lesions in group I that were fatty and occurred almost solely in small, never in large arteries, the arterial lesions in group II were proliferative and without fatty change and occurred in many large and medium arteries in addition to small arteries. Some of the arterial changes in group II resemble those of human coronary arteriosclerosis without fatty change.

In group III 90% of the arterial lesions were fatty-proliferative. In their proliferative character and occurrence in many large and medium arteries, these lesions were unlike those in group I but obviously similar to those in group II. Inasmuch as rabbits in both groups II and III were similarly injected with horse serum, it is reasonable to infer that (a) in the absence of a large amount of lipid in blood, sites of allergic arterial injury evolved into proliferative lesions in group II, and (b) in the presence of a large amount of lipid in blood, sites of allergic injury evolved into fatty-proliferative lesions in group III. The inference is supported by the observation that in group I elevation of serum lipids,
similar to that in group III, induced in the absence of allergic arterial injury lesions that were only fatty and limited almost solely to small arteries, never large arteries.

In thickened intima in advanced fatty-proliferative arterial lesions of group III there was commonly, as is often the case in human atherosclerosis, lack of or little elastification and instead fatty-hyaline change. In contrast, elastification, sometimes marked, was often prominent and fatty-hyaline change was absent in thickened intima in proliferative arterial lesions in group II.

Arteries of man are uncommonly subject to as intense injury and the amount of lipid in blood is usually not as great as that in rabbits in these experiments. Consequently, coronary arterial lesions induced in these relatively short term experiments might not be expected to resemble coronary athero-arteriosclerosis in man, where arterial reaction may occur to injury that is less intense and perhaps recurrent at longer intervals or more protracted over a period of many years. However, as shown in comparative illustrations, some of the fatty-proliferative changes in coronary arteries of rabbits in group III closely resemble the fatty-proliferative changes that in some cases constitute coronary athero-arteriosclerosis in man: presence of lipid filled "foam" cells distributed throughout or clustered deep in thickened intima, sometimes overlying fragmented and/or reduplicated internal elastic membrane, presence of proliferating cells over and around such atheromatous areas, frequent lack or small amount of elastification of thickened intima, fatty-hyaline intimal change, foci of medial degeneration in which lipid sometimes occurs in medial smooth muscle cells, and cellular proliferative and infiltrative change and fibrosis in adventitia. It is, therefore, reasonable to suggest that these fatty-proliferative arterial lesions in rabbits and certain of those in man evolve through similar stages by similar mechanisms.

Comparison of the earlier and the advanced fatty-proliferative arterial lesions in the rabbits provides information about the evolution of the latter, and in turn permits certain inferences about the pathogenesis of athero-arteriosclerosis in man. In the earlier lesions many proliferating intimal cells became filled with lipid. This fatty-proliferative intimal change often overlay fragmented internal elastic membrane and altered media. In advanced lesions the frequent lack of or little elastification of thickened intima and the presence, instead, of a kind of fatty-hyaline change may have been due to inhibition of the normal reparative function (which may include synthesis of elastic fibers) of proliferating intimal cells that become burdened with lipid. These findings and the interpretation concerning inhibition of reparative function of proliferating intimal cells that become lipid filled are in agreement with those of others (40) concerning arterial injury produced by freezing in rabbits fed a diet rich in cholesterol. In the present experiments, proliferating cells that were either less or not burdened with
lipid sometimes appeared to have formed a jacket or cap over or around clusters of lipid laden "foam" cells. Thus the latter became trapped, often deep in the thickened intima near or at sites of fragmentation and/or reduplication of the internal elastic membrane.

Degenerative changes in the media-internal elastic membrane region are commonly evident in athero-arteriosclerosis and, as recently emphasized (41), are probably important to its development. In arteries of rabbits in groups II and III degenerative changes occurred in the media-internal elastic membrane region. Data obtained from clinical observation and from experiment indicate that these changes probably resulted from allergic injury in this region (42-48). The proliferation of cells and fatty-hyaline change in arteries of animals in group III were probably reactions to allergic injury that were modified by lipid from the blood. Proliferation of cells, infiltration of inflammatory cells, and in time fibrosis in the adventitia probably also resulted from this injury. In this connection it is perhaps pertinent that foci of small round cells, probably representing chronic inflammatory cells, and fibrosis in adventitia not uncommonly occur in human athero-arteriosclerosis in youth (49) as well as older age.

In many of the proliferating cells in arterial intima in hearts of rabbits in groups II and III and in some lipid rich "foam" cells in intima and subjacent media in hearts of rabbits in group III nuclei with caterpillarlike chromatin pattern or owl eye appearance were present. Such nuclei have previously (50) and here been shown to occur in some reacting smooth muscle cells in rabbit and human hearts. They have also been demonstrated to occur in normal immature and normal appearing and reacting mature striated muscle cells and fragments of the latter in human hearts (50, 51). These observations indicate, in harmony with observations made by others (15, 52-56) with either the light or electron microscope, that at least many of the cells, including "foam" cells, in thickened intima in experimentally induced and naturally occurring coronary atheroarteriosclerosis are smooth muscle cells that evolved in reparative reaction to arterial injury.

The yellow-white fatty streaks and plaques that developed in the aortas of all rabbits fed the cholesterol supplement (groups I and III) were most marked in the thoracic portion. The amount of aortic fatty change was considerably greater in group III that was fed the cholesterol supplement and concomitantly received foreign serum than in group I that was fed the cholesterol supplement but received no foreign serum. A striking feature of the fatty change in the aorta was its invariably abrupt termination within the mouths of the coronary arteries at their juncture with the heavily elastic aorta. Prominent reparative change like that which occurred in proliferative and fatty-proliferative arterial lesions did not occur in either the fatty lesions of the aorta or those of small arteries.
The primary purpose of including two illustrated cases of human coronary athero-arteriosclerosis in this report is to provide material for morphologic comparison of fatty-proliferative changes experimentally induced in coronary arteries of rabbits with those in naturally occurring coronary athero-arteriosclerosis in man. Certain comments concerning the nature of these human cases appear to be pertinent to the experimental investigation here reported. One of the patients (Fig. 3) died with advanced rheumatic heart disease with calcific stenosis and insufficiency of the mitral and aortic valves. The other (Figs. 7, 8, and 12) died with severe chronic proliferative and membranous glomerulonephritis associated with disseminated lupus erythematosus. Since childhood he had experienced repeated attacks of asthma and many episodes of urticaria. Allergy is probably an important causative factor in rheumatic cardiovascular disease (16, 23-27) and in the cardiovascular disease of disseminated lupus erythematosus (30, 57-65). Furthermore, it has long been known that rheumatic injury to coronary arteries occurs and in some cases leads to sclerosis of these vessels (9-19); and clinico-pathologic observations indicate that injury to coronary arteries caused by disseminated lupus erythematosus also occurs and can lead to sclerosis of these arteries (66, 67).

The occurrence of coronary atherosclerosis with advanced rheumatic heart disease in one of the human cases considered in this report and with advanced glomerulonephritis associated with lupus erythematosus in the other case may be fortuitous. It is possible, however, that in both cases repeated or protracted allergic injury to coronary arteries and local changes in reaction to the injury favored repeated or protracted deposition and accumulation of blood-borne lipid in the vessel walls, and thus lead to athero-arteriosclerosis. This possibility is supported by results of the experimental investigations here reported.

SUMMARY

In rabbits that received a dietary supplement of cholesterol, 0.5% by weight, and concomitant injections of horse serum (group III) over a period of 80 days, coronary arterial lesions developed that in the main were different in quality and distribution from those in rabbits that received the cholesterol supplement alone (group I), and of different quality from but in distribution similar to those in rabbits that received horse serum alone (group II).

Fatty lesions developed in small, rarely in medium, but never in large arteries of rabbits in group I, and these changes do not resemble coronary athero-arteriosclerosis in man. Proliferative lesions without fatty change developed in large, medium, and small arteries of rabbits in group II, and some of these closely resemble human coronary arteriosclerosis without fatty change. The changes that developed in large, medium, and small arteries of rabbits in group III were in very large majority fatty-proliferative lesions. Some of these closely
resemble the changes that in some cases constitute coronary athero-arteriosclerosis in man.

Nuclei with caterpillarlike chromatin pattern in longitudinal section and owl eye appearance in transverse section were observed to occur in many of the proliferating cells in thickened arterial intima in hearts of rabbits in groups II and III and in some of the lipid rich "foam" cells in arterial intima and subjacent media in hearts of rabbits in group III. Such nuclei have been observed to occur in some reacting smooth muscle cells and normal immature and reacting mature striated muscle cells of the heart. These observations indicate that at least many of the cells, including "foam" cells, in thickened intima in the experimentally induced and in naturally occurring coronary athero-arteriosclerosis are smooth muscle cells that evolved in proliferative reaction to arterial injury.

Fatty change developed in aortas of the rabbits in groups I and III, and was significantly greater in group III.

Results of this investigation support the hypothesis that the synergy of allergic injury to arteries and lipid-rich diet can lead to athero-arteriosclerosis.

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EXPLANATION OF PLATES

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FIG. 1. Right coronary artery of rabbit 10-316 (group II) that received a diet of Rockland rabbit pellets without cholesterol supplement and 2 large injections of horse serum spaced 17 days apart. The animal was sacrificed 7 days later. Proliferation of smooth muscle cells in media and in intima. Straightening of internal elastic membrane on the left. Weigert–hematoxylin and eosin. × 153.

FIG. 2. Left coronary artery of rabbit 97-64 (group III) that received a diet of cholesterol-coated pellets for 82 days and concomitantly 4 large injections of horse serum spaced 17 days apart. The animal was sacrificed 1 day later. Fatty-proliferative changes comprising proliferation of intimal cells and distension of intimal and medial cells with lipid. In right lower portion of artery dense accumulation of lipid-laden cells deep in intima overlies fragmented internal elastic membrane and focus of degenerative changes in the media in which many smooth muscle cells are filled with lipid. Weigert–hematoxylin and eosin. × 96.

FIG. 3. Right coronary artery of a 35-yr-old man who died with calcific stenosis and insufficiency of the mitral and aortic valves, marked athero-arteriosclerosis of right and left coronary arteries, and old and recent infarcts of interventricular septum and lateral wall of left ventricle (autopsy 21154, The New York Hospital). Fatty-proliferative changes comprising proliferation and distension of intimal cells with lipid. Dense accumulation of lipid deep in intima overlies fragmented internal elastic membrane. Focal degenerative changes, including fatty change, in media. Note resemblance of arterial changes to those in the rabbit coronary artery shown in Figs. 2 and 4. Hematoxylin and eosin. × 120.

FIG. 4. Higher magnification of the right lower portion of a close serial section of the rabbit artery shown in Fig. 2. Dense accumulation of lipid-laden cells deep in intima overlies fragmented internal elastic membrane and degenerative changes in medial smooth muscle cells. Note resemblance of arterial changes to those in the human coronary artery shown in Fig. 3. Weigert-hematoxylin and eosin. × 153.
(Minick, Murphy, and Campbell: Induction of athero-arteriosclerosis)
FIG. 5. Left coronary artery of rabbit referred to in Figs. 2 and 4. Fatty-proliferative intimal and medial changes resembling those seen in the human coronary artery in Figs. 7 and 8. In right central portion of artery dense accumulation of lipid deep in intima overlies fragmented internal elastic membrane and degenerative medial changes with lipid-laden smooth muscle cells. Overlying the dense accumulation of intimal lipid is a fibro-cellular cap containing foam cells. Weigert-hematoxylin and eosin. × 75.

FIG. 6. Higher magnification of right central portion of rabbit artery shown in Fig. 5. Fibro-cellular cap containing foam cells overlies dense accumulation of lipid deep in intima. This accumulation overlies altered internal elastic membrane and degenerative changes in media in which many smooth muscle cells contain lipid. Note resemblance of these arterial changes to those in the human coronary artery shown in Fig. 8. Weigert-hematoxylin and eosin. × 120.

FIG. 7. Right coronary artery of a 19-yr-old man who died with severe chronic proliferative and membranous glomerulonephritis, probably a manifestation of disseminated lupus erythematosus. There was pronounced athero-arteriosclerosis of coronary arteries, especially the right (autopsy 21578, The New York Hospital). Fatty-proliferative intimal and medial changes closely resembling those in the rabbit coronary artery shown in Figs. 5 and 6. In middle and central lower portion of figure dense accumulation of lipid deep in intima overlies fragmented and reduplicated internal elastic membrane. Degenerative changes in media in which many smooth muscle cells contain lipid. Verhoeff's elastic tissue stain. × 75.

FIG. 8. Higher magnification of middle and central lower portion of a close serial section of the human artery shown in Fig. 7. Dense accumulation of lipid deep in intima overlies altered internal elastic membrane and degenerative changes in media in which many smooth muscle cells contain lipid. Note resemblance of these arterial changes to those in the rabbit coronary artery shown in Fig. 6. Verhoeff's elastic tissue stain. × 120.
(Minick, Murphy, and Campbell: Induction of athero-arteriosclerosis)
FIG. 9. Aorta and right coronary artery of rabbit 97-62 (group III) that received a diet of cholesterol-coated pellets for 82 days and concomitantly 4 large injections of horse serum spaced 17 days apart. The animal was sacrificed 1 day later. The predominantly fatty change in the aorta terminates abruptly in the mouth of the coronary artery at its juncture with the heavily elastic aorta. The aortic change lacks the prominent reparative features of the fatty-proliferative changes in the coronary artery in lower part of this figure which is shown at higher magnification in Fig. 10. Figs. 22, 24, and 25 are also from this animal. Verhoeff’s elastic tissue stain. × 40.

Fig. 10. Higher magnification of the rabbit coronary artery shown in lower part of Fig. 9. Fatty-proliferative intimal thickening with fatty-hyaline change and numerous foam cells. Fragmentation and reduplication of internal elastic membrane. These arterial changes closely resemble those in the human coronary artery shown in Fig. 12. Verhoeff’s elastic tissue stain. × 120.

Fig. 11. Right coronary artery of rabbit 10-011 (group III) that received a diet of cholesterol-coated pellets for 78 days and concomitantly 3 large injections of horse serum spaced 17 days apart. The animal died 1 day after the last desensitizing injection. Fatty-proliferative intimal thickening with foam cells. In upper portion of figure note the obvious bundles of smooth muscle extending from just beneath the endothelium about halfway to the internal elastic membrane. Lipid in some medial smooth muscle cells. Weigert–hematoxylin and eosin. × 192.

Fig. 12. Right coronary artery of the man referred to in Figs. 7 and 8. Fatty-proliferative intimal thickening with fatty-hyaline change and many foam cells. Straightening, fragmentation, and reduplication of internal elastic membrane. These arterial changes are strikingly like those in the rabbit coronary artery shown in Fig. 10. Hematoxylin and eosin. × 96.
(Minick, Murphy, and Campbell: Induction of athero-arteriosclerosis)
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Fig. 13. Branch of left coronary artery of rabbit 97-29 (group III) that received a diet of cholesterol-coated pellets for 79 days and concomitantly 4 large injections of horse serum spaced 17 days apart. The animal was sacrificed 6 days later. Fatty-proliferative changes comprising clusters of foam cells in intima with overlying fibrocellular caps. Internal elastic membrane fragmented at many points. Occasional foam cells in media which is focally thinned. Weigert-hematoxylin and eosin. × 153.

Fig. 14. Higher magnification of changes similar to those in Fig. 13 in a branch of left coronary artery of rabbit 10-013 (group III) that received a diet of cholesterol-coated pellets for 81 days and concomitantly 4 large injections of horse serum spaced 17 days apart. The animal was sacrificed 8 days later. Fatty-proliferative changes include distension with lipid of cells on intimal side of (to left) and at the sites of break in internal elastic membrane. At one of these sites is a foam cell which contains a nucleus with caterpillarlike chromatin pattern. Weigert-hematoxylin and eosin. × 768.

Fig. 15. Branch of left coronary artery of rabbit 96-96 (group II) that received a diet of Rockland rabbit pellets without cholesterol supplement and 6 large injections of horse serum and 6 large injections of swine serum over a period of 6 months. The animal was sacrificed 3 days later. This figure and Fig. 16 are from the artery shown in Figs. 21 and 23. Caterpillarlike chromatin pattern in nuclei of intimal cells is like that in medial smooth muscle cells in lower half of this figure, in lower portion of Fig. 16, and in Figs. 19 and 20. Verhoeff's elastic tissue stain. × 1200.

Fig. 16. Rabbit artery referred to in Figs. 15, 21, and 23. Structure of caterpillar nuclei in intimal cells above the internal elastic membrane is like that in the medial smooth muscle cells below it. Verhoeff's elastic tissue stain. × 1200.
(Minick, Murphy, and Campbell: Induction of athero-arteriosclerosis)
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FIG. 17. Branch of left coronary artery of rabbit 99-06 (group III) that received a diet of cholesterol-coated pellets for 83 days and concomitantly 4 large injections of horse serum spaced 17 days apart. The animal was then maintained on a diet of pellets without cholesterol supplement for 60 days and then sacrificed. Late fatty-proliferative changes with marked narrowing of lumen. Fibro-cellular cap or jacket above and around foam cells and fatty-hyaline change deep in intima. Figs. 18 to 20 are also from this animal. Hematoxylin and eosin. × 192.

FIG. 18. Higher magnification of a segment of the left upper portion of Fig. 17. Fatty-hyaline change in intima. Some intimal cells, including a foam cell, have nuclei with distorted owl eye or caterpillarlike chromatin pattern. Small focus of granules of calcium just above center of figure. Giemsa. × 615.

FIG. 19. Higher magnification of left lower portion of artery shown in Fig. 17. Fatty-hyaline change in intima. Some intimal cells, including foam cells, have nuclei with distorted owl eye or caterpillarlike structure. Many caterpillar or owl eye nuclei in medial smooth muscle cells. Hematoxylin and eosin. × 480.

FIG. 20. Higher magnification of lower portion of media shown in Fig. 19. Caterpillarlike or owl eye chromatin pattern in medial smooth muscle cells. Hematoxylin and eosin. × 1200.
(Minick, Murphy, and Campbell: Induction of athero-arteriosclerosis)
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Fig. 21. Large branch of left coronary artery of rabbit (group II) referred to in Figs. 15, 16, and 23. Late proliferative changes with marked musculo-elastic intimal thickening. A large proportion of the cells in media and intima have caterpillar or owl eye nuclei (Figs. 15, 16, and 23). The internal elastic membrane is focally straightened and fragmented. Verhoeff’s elastic tissue stain. × 75.

Fig. 22. Large branch of left coronary artery of rabbit (group III) referred to in Figs. 9, 10, 24, and 25. Late fatty-proliferative changes with marked intimal thickening. Foam cells deep in intima and in media. Dense fibro-cellular caps overlie accumulations of foam cells deep in intima. Verhoeff’s elastic tissue stain. × 120.

Fig. 23. Higher magnification of left lower portion of artery shown above in Fig. 21. Proliferation of cells deep in intima. The nuclei of almost all of these cells have a caterpillarlike chromatin pattern or owl eye appearance like those in proliferating medial smooth muscle cells (Figs. 15, 16, 19, and 20). Verhoeff’s elastic tissue stain. × 418.

Fig. 24. Higher magnification of left lower portion of artery shown above in Fig. 22. Deep in the intima lying just above fragmented internal elastic membrane is a focus of foam cells, some of which have distorted owl eye or caterpillar nuclei. Overlying this is a dense fibro-cellular cap in which nuclei of some cells have caterpillarlike chromatin pattern. Verhoeff’s elastic tissue stain. × 384.
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Fig. 25. Right coronary artery of rabbit (group III) referred to in Figs. 9, 10, 22, and 24. Fatty-proliferative changes with straightening, fragmentation, and reduplication of internal elastic membrane. Note the similarity of the presence of musculo-elastic hyperplastic change and the lack of foam cells in the left lower portion of this artery to the changes in the artery shown in Fig. 26. Note also the difference between the presence of the fatty-hyaline change in the remainder of this artery and the absence of such changes in the artery in Fig. 26. The areas of most marked fatty-hyaline change are the areas of least elastic hyperplastic change. Some medial smooth muscle cells, especially in the region subjacent to the most markedly fragmented portion of internal elastic membrane, are filled with lipid. Verhoeff’s elastic tissue stain. X 153.

Fig. 26. Right coronary artery of rabbit 10-057 (group II) that received a diet of Rockland rabbit pellets without cholesterol supplement and 4 large injections of horse serum spaced 17 days apart. The animal was sacrificed 7 days later. Proliferative change with marked musculo-elastic hyperplastic intimal thickening and straightening and focal fragmentation of internal elastic membrane. Weigert-hematoxylin and eosin. X 192.

Fig. 27. Small myocardial artery of rabbit 10-045 (group I) that received a diet of cholesterol-coated pellets for 80 days, then Rockland rabbit pellets without cholesterol supplement for 60 days. The animal was then sacrificed. No injections of horse serum were given. Distension of intimal and medial cells with fat. Hematoxylin and eosin. X 192.
(Minick, Murphy, and Campbell: Induction of athero-arteriosclerosis)