1965

Role of inflammation in atherosclerosis

Donald Eugene Freeman
University of Nebraska Medical Center

ROLE OF INFLAMMATION IN ATHEROSCLEROSIS

Donald Eugene Freeman

Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine

College of Medicine, University of Nebraska

February 1, 1965

Omaha, Nebraska

TABLE OF CONTENTS

Role of Inflammation in Atherosclerosis

INTRODUCTION

The phrase, "You're only as young as your arteries," commonly used today, may contain some element of truth in light of the large numbers of deaths and high morbidity from myocardial infarction, cerebral thrombosis, and from diabetic gangrene in the United States. Degenerative arterial disease in the form of atherosclerosis is often alarming in the abruptness of its onset or in the degree of its progression. Plaques in coronary and cerebral arteries often ulcerate to cause thrombosis or obstruct by slowly developing stenosis. Vascularized plaques are known to become hemorrhagic, and lipid deposits extending into the media have weakened vessels with resulting aneuryisms.[1] Grossly, visible atherosclerosis appears as variably-sized patches of white, curdlike elevations along the luminal wall. Microscopically, lesions are initially localized to the intima and consist of large extracellular deposits of cholesterol; later the media becomes involved and in some cases calcification occurs.

The pathogenesis of atherosclerosis is receiving widespread attention at present from many researchers. Out of a voluminous literature their tenets can be placed into two divergent schools: the "Thrombotic Encrustation" hypothesis of Duguid (1957)[2] and Astrup (1959)[3] and the "Filtration" hypothesis of

Page (1940)[4] and Adams (1963)[5]. In the former school much attention has been given to thrombin formations of all sizes from microscopic to occlusive which become incorporated into the intima by actively growing endothelium. These formations are reported to produce fibrosis and vascularization acting as a nidus for a chronic course of breakdown and repair. The filtration school regards the serum that passes through the intima as the primary source of lipid in the atheroma. In some manner small fractions of serum become insoluble and filter off against the internal elastic membrane. Low intensity oxidative polymerization of lipoprotein and a production shutdown of phospholipid are considered important.

Atherosclerosis recurs at locations on the arterial tree that sustain more of the daily stress and strain from hemodynamic injury. In 1961 enzyme studies led to the concept of induced histamine whereby cells in damaged areas synthesize minute amounts of histamine to act as an inflammatory mediator in cell repair.[6, 7] Since endothelial cells phagocytize avidly under histamine stimulation,[8] the "Filtration" hypothesis includes hemodynamic trauma to endothelial cells as a cause for induced histamine production and as a reason for increased perfusion of the underlying intima with serum lipoprotein. Serum breakdown products then becoming extracellular in the intima by their irritative properties would induce further histamine

production maintaining a stimulus to the endothelial cells and adding intimal inflammation to the extent of granuloma formation. Harman[9, 10] tested chlorpheniramine and Dury[11] tested cortisone in rabbit atheroma and kept the process under moderate control supporting the idea of a role for inflammation.

If the assumption is allowed that the ability to respond to tissue injury is uniform through the body, then the degree of inflammatory capability in the aortic wall could be considered to parallel that in the viscera, muscles, and skin. Thus, if inflammation in the arterial wall contributes to atherogenesis, then those patients with a clinical history of coronary atherosclerosis might show a greater response to a skin injury than those people with a negative cardiac history. This possibility was evaluated by following the development of the inflammatory response to a standard skin abrasion in white males with and without a history of myocardial infarction.

METHODS AND RESULTS

Male volunteers ages 36 to 59 years had the Rebuck skin window[12] applied to their forearms. The total number came to 47 men; clinically demonstratable myocardial infarction had occurred in 19 of these at least six months before. Occupation, health, diet, habits, etc. were not used as a standard for selection; yet all those tested were gainfully employed.

After the skin from the flexor surface of the left forearm was cleaned with 70% ethyl alcohol and Virac, a sterile scalpel blade no. 15 was drawn gently across the surface with quick back-and-forth movements abrading the epidermis (not cutting) in an area about 1½ by 1½ cm. The base of the lesion was made to sufficient depth that the dermis could be seen with oozing blood and serous fluid. Two abrasions were made in each test. Glass coverslips were pressed onto the abrasions, the time was noted, and a light dressing was taped on to hold the coverslips in place. At four hours the entire dressing was changed with discarding of the old one. At nine hours the dressing was carefully removed, and the patient was dismissed. The coverslips were prepared for microscopic inspection in the same manner as are hospital blood smears . . . Wright's or Trichrone dye "fixed" the cells for three minutes; then with distilled water added approximately 1:1, the dye stained the cells for another three minutes. After a tap water rinsing the coverslips were dried in air and mounted on clean regular-sized microscope slides.

Under the light microscope low power scaning often showed that several thousand cells were adherent. At 450 power intracellular detail was clearly visible in most areas; the cell population was composed chiefly of neutrophils (segmented forms) and macrophages with innumerable pseudopod formations that probably represented active phagocytosis. A typical focus of

cells on a coverslip contained the majority of neutrophils packed so tightly in the center that cytoplasmic borders were almost indistinguishable. Macrophages tended to form a ring around the neutrophils being more loosely packed on the outside. On some slides this pattern was minimal with apparent mixing of the cell types. Two hundred cells were individually inspected and the cell types were classified as the percentage of macrophages present from each coverslip. Only rarely was a small lymphocyte seen although large and atypical lymphocytes were present in a definite percentage being counted as macrophages. (See Table 1a and 1b, pages 6 and 7) The non-heart attack volunteers had macrophage percentages averaging 25.4 ± 11; the heart attack group percentages averaged 39.0 ± 19. The T value of the difference between the groups is 2.71; the P value is less than 0.01 using 47 degrees of freedom.

The data seems to confirm the references to inflammation made earlier. In quick review the inflammatory process was: 1. reported to be a factor in atherogenesis, 2. deduced to occur in skin, muscles, and viscera with corresponding intensity to its occurrence in the arterial wall, 3. proposed to be more intense in those who suffered myocardial infarction at a relatively young age. However, the subjectiveness of microscopic evaluation even when the examiner exerts every effort to be impartial will and should lead the reader to careful scrutiny of any data or

HEART ATTACK GROUP

Book No.	Age	Time	Macrophage Percentages
6	48	8:45	12
13	46	8:50	28
18	52	8:55	38
24	43	8:00	21
25	53	9:00	71
26	50	9:00	52
32	51	9:00	51
33	59	8:20	41
35	44	7:45	48
36	45	8:40	93
39	36	9:20	28
41	48	8:25	58
43	45	8:45	28
44	49	9:40	27
46	49	8:00	33
48	48	9:30	26
52	43	9:30	17
53	45	9:10	39
56	54	9:00	27
Total number= 19	$\frac{x}{n}$ = 47.8 yrs.		$\frac{x}{n}$ 39.0 ± 19 standard deviation

Table 1a

NON-HEART ATTACK VOLUNTEERS

Book No.	Age	Time	Macrophage Percentages
4	46	9:10	41
5	57	9:30	14
7	42	8:55	8
8	41	8:40	25
9	47	9:15	26
10	46	9:25	20
11	47	9:30	40
12	51	9:20	50
14	40	8:55	22
15	44	9:05	19
16	49	8:45	16
17	46	8:45	25
19	47	8:50	48
21	41	8:50	27
20	43	9:20	17
22	46	9:30	25
23	41	8:55	19
30	40	9:00	35
2	50	9:12	25
34	55	8:30	23
37	36	9:15	29
38	36	7:35	16
40	44	9:10	6
42	46	8:55	50
45	42	9:15	31
47	49	8:30	26
50	43	9:45	36
51	48	9:10	28
Total 28	$\frac{x}{n}$ = 44.5 yrs.		$\frac{x}{n}$ = 25.4 $\pm$ 11 standard deviation

Table 1b

conclusions. For this reason the slides are to be submitted for a second and possibly a third evaluation by competent microscopists, and plans are being made* to enlarge the present series in the summer of 1965.

DISCUSSION

The concepts of atherogenesis behind the experiment will be discussed in three parts - A, B, and C:

Part A

Inspection of human and experimental atherosclerosis finds the process more often localized into discrete plaques that have a predilection for the aortic arch and its branches, at bifurcations, in tortuous vessels, and in coronary arteries in the sections running outside the myocardium.[13, 14] Both schools agree here: local factors would seem prominent in inducing and/or maintaining the process.[2, 10]

Duguid (1926)[15] suggested the aorta in systoli expands not only in diameter but in length with a resultant stretch on branch arteries like the intercostals, which are fixed to the tissues. Small plaques regularly occur at the lower margins of these ostia in the earliest stages of the process. (See Figure 1)

*Denham Harman, Chair of Cardiovascular Research-Department of Biochemestry, University of Nebraska College of Medicine.

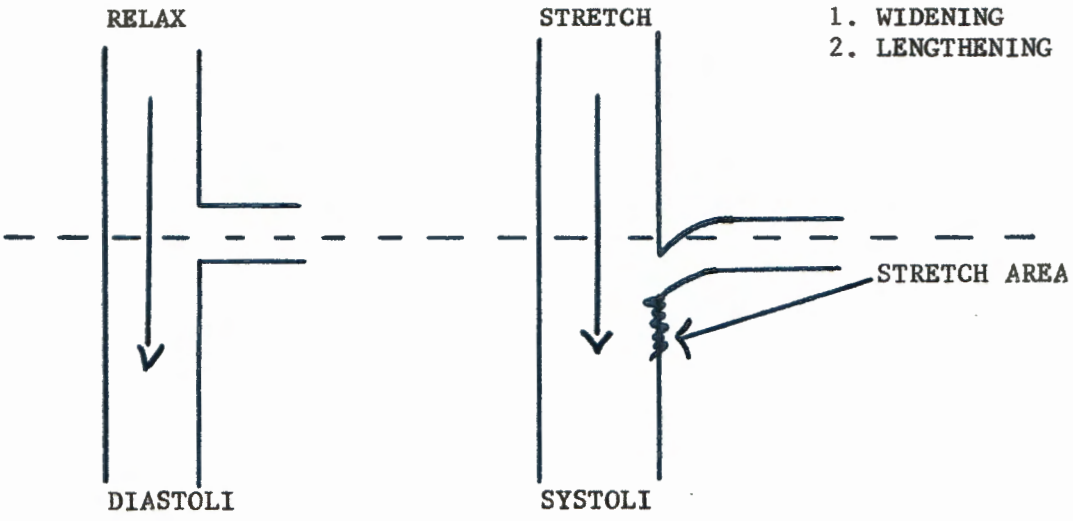


Figure 1

Laplace's Law*[14] applied to vascular hemodynamics suggests that blood pressure opposed by surrounding tissue resistence along with the radius of the vessel and the factor of curving primarily determine wall flexibility. It seems that the areas of the arterial trees sustaining more daily stretch or flexion have a proportionally greater degree of atherosclerosis. Rabbits fed cholesterol underwent lumbar sympathectomy with loss of artery contractility in the right leg. The flexibility was noticeably accentuated in these arteries as was the atherosclerosis; a second group of rabbits whose medium-sized arteries became rigidly calcified after induction of necrosis with calciferol to prevent flexion also ate the cholesterol diet but had minimal

*Leplace's Law states that T (total tangential tension in dynes/cm. length of vessel) = Pr (pressure as dynes/cm.^{2})(radius in cm.)

signs of the process in the sympathectomized leg.[16] A common example in man is the rarity of the process in the wall of the internal carotid artery where it passes through the carotid canal of the skull. This artery is notoriously susceptable to plaque formations but seems to be immune in the area that is surrounded by bony framework where it cannot distend widely. Laplace's Law explains that the aortic arch sustains the highest tangential tentions of any artery on the basis of the widest radius, maximal hydrostatic pressure at this location, and its curvature. Yet, while the aortic ring is the first location for fatty involvement, larger areas of fatty change and greater fibrosis occur in its descending thoracic and abdominal portions.[1] Perhaps the arch of the aorta has more firm external support than the more distal aorta because of the mediastinum?

The effect of laminar flow based on Bernoulli's Theorem* is also regarded in this connection.[13] Speeding up of the bloodstream with each systoli should periodically diminish support to the intima pressing and releasing force over and over. Slight compressions and expansions continued over a lifetime might then cause atherosclerosis especially at bifurcations, curves, and branch points.

*Bernoulli's Theorem states that fluid possesses energy as velocity and pressure; as kinetic energy increases the (hydro) static pressure decreases. In tubular flow, as energy is shifted into propelling fluid, hydrostatic pressure supporting the vessel wall is lessened.

The veins and pulmonary vessels usually enjoy exemption from intimal crippling because as Laplace's Law explains, tangential tension is minimal when hydrostatic pressures are low. However, Bernoulli's Theorem would hold onto the idea that slow-moving blood in a volume system better supports the intima with a higher static pressure. Pulsation is absent in veins and present in pulmonary arteries. In pulmonary hypertension of severe intensity sustained over an adequate time period, atherosclerosis can be found in elastic arteries 1.0 mm. or more in diameter.[17] Notwithstanding some confusion about the facets of wall flexibility and intimal support which might be important, the key idea is that rhythmic distention and recoil of the vessel in cadence with each heartbeat day after day for a lifetime in some manner is thought to be a factor in atherogenesis.

Part B -- Thrombotic Encrustation in the Pathogenesis of Atherosclerosis

Duguid studied healed, recanalized coronary thromboses[18, 19, 20] and reported finding many microscopic thrombi on the aorta, coronaries, and cerebral arteries in statistically significant numbers of human autopsies. From these studies he hypothesizes in detail in 1957 that fibrin thromboses of microscopic size daily form along the walls of arteries, and the versatile endothelium, always ready for active growing, immediately cover up the small deposits of fibrin. An occluding thrombus will recanalize, if survival permits, and even a broad surface of fibrin will be

soon separated from the blood by a canopy of endothelium. Once incorporated into the intima Duguid deduced that necrosis occurs, especially in the centers of large thrombi, and fibrous connective tissue forms as an aftermath of vascularization. The fibrous tissue seems at home in the healing intima and was commented upon as "fibrous hyperplasia" by him in 1926.[15] Taylor,[21] in a 10-year study of aortic repair after freezing in rabbits, said healing occurs by fibroblastic proliferation without inflammation; healing is done so remarkably well, if the vessel is not repeatedly molested, that regenerated intima is almost indistinguishable from undamaged vessel. An electronmicroscope study by Levene[22] supports the thrombus etiology idea because only fibers resembling fibrin were seen in plaques and normal intimas despite the fact that these same tissues took collagen stains. To reinforce his tenets Duguid cites Harrison,[23] Heard,[24] and McLetchie,[25] who separately injected thrombogenic materials into the pulmonary arteries of rabbits. At necropsy gross and microscopic sections of the arteries repairing themselves after a bombardment of intramural thrombi were found strickingly similar to the various stages of human atherosclerosis. Duguid cites Crawford and Levene[26] for their study of 100 human autopsies and their complete agreement that the organized subendothelial thrombus plays a major role. Geiringer[27] had proof that the normal intima is avascular, acquiring a secondary blood supply

only, if it thickens 0.5 mm. or more for the aorta or 0.35 mm. for the proximal anterior descending branch of the left coronary artery. Further he claimed a thrombus newly formed on the luminal endothelium could easily cause simple infarction of the underlying intima.

Duguid was aware of the work of Winternitz[28] and Paterson[29] (1938) when he attended a demonstration by Morgan[30] at the Great Britain Pathological Society Meeting in 1956. In a classic monograph* Winternitz had clear photomicrographs to visibilize the complexity of the human vasa vasorum as local vascular adjustments were made to stress. A thickened intima was shown to acquire a secondary blood supply both from extrinsic arterioles perforating from the media and from intrinsic capillaries feeding off luminal blood, the so-called "high pressure capillaries." Paterson had attested that the intrinsic mural hemorrhages were common in coronary thromboses at autopsy for the first time connecting hemorrhage with the process. At the meeting Morgan explained that cholesterol and other fatty debris in the atheroma are products of blood breakdown <u>in situ</u> recurring over long periods.

The role of hemorrhages was set forth by Paterson (1939),[32] (1956),[33, 34, 35] (1957),[36] (1961)[37, 38] from a large series of autopsies: small capillaries surround the encrusted thrombus in the intima and under the stretch and recoil of blood pressure and hypertension from exercise are broken periodically exuding small

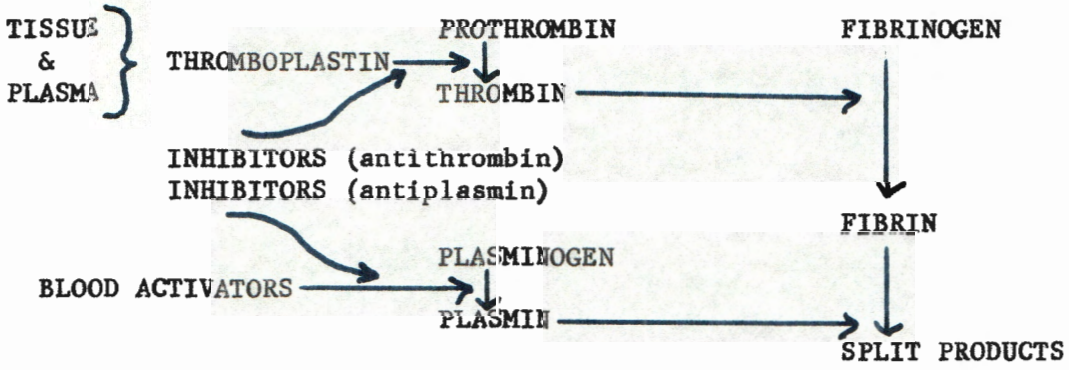
*Cited by Campbell Moses[31] Atherosclerosis, Mechanism as a Guide to Prevention.

deposits of blood. An organizing thrombus, even a small one, cannot move in an elastic fashion with the vessel wall and consequently tears away from the fibroblasts and fibrin of healing. They perseveringly reattach but not until minute hemorrhage has occurred at the breakpoints. Chandler[39] pointed out that platelets are rich in cholesterol and displayed their *in vitro* phagocytosis by monocytes in his report. Duguid[20] had written that rbc's might be a good source of lipids via thrombi. Willis[40] corroborated this role by finding intrinsic hemorrhage in 55 out of 123 of coronary thrombosis in his autopsies. More,[41] Movat,[42] Haust[43, 44] also felt early plaques were made up of recently formed thrombi and fibrin with enmeshed blood cells and platelets. Horn (1936)[45] and Heard[46] from 100 and 25 autopsies respectively affirm that hemorrhage was not seen in normal intimas which corresponds to the concept of the intima as an avascular tissue. Thus, hemorrhage is a suppliment to the process not an initiating factor; local trauma predisposes to atherosclerosis; fibrin deposits easily traumatize surrounding tissue as the vessel stretches and recoils.

In 1948 Mole[47] was investigating cadaver blood searching for the reason why this blood is usually clotted in the larger vessels but remains fluid in peripheral ones. He discovered that blood in small vessels is devoid of fibrinogen and high in fibrinolysin and concluded the later material more easily saturated non-circulating

blood because it is produced by the dying endothelium.

T. Astrup (1956)[48, 3] testified that there exists a dynamic equilibrium between the system that forms fibrin and a system of fibrinolysis (see Figure 2) and that the intima has potent



(Figure 2)

thromboplastic activity but little plasminogen; the adventitia is just opposite in its endowments while the media lies in the middle and shares some activity of each. His notion of thrombus organization requires complete dissolution of the clot by fibrinolysis before migrating fibroblasts can proliferate in its substrate. If the fibrinolytic system is of low potency (due to a low concentration of activating agents or high concentrations of inhibitors), organization will be delayed and many fibroblasts will migrate around fibrin. Later when resolution finally happens, excessive connective tissue will be present. In the fibrinolysin poor intima and in the necrotic centers of thrombi, fibrin and necrotic debris persist for an abnormally long time to account for the

atheroma* of atherosclerosis and eventually its fibrous hyperplasia.

Fibrin is "stickey,"[48] and newly formed still intraluminal fibrin catches rbc's and platelets while imbibing serum lipids before incorporation; the minute hemorrhages of Paterson could account for a steady supply of serum and formed elements of the blood, both rich in lipid. Calcification[48] is facilitated by release of alkaline phosphatase from disrupted connective tissue.

Astrup explains the immunity of veins and the pulmonary system on the basis of abundant vasa vasorum enabling sizeably more fibrinolytic activity. Todd[49] vouches for the higher fibrinolytic potency veins, sinusoids, and pulmonary vessels over arteries in his 1958 study.

In summary, Duguid's, Astrup's, and Patterson's ideas in stepwise fashion are:

1. A fibrin thrombus, any size from gross to microscopic, forming on the endothelium can entangle red blood cells, platelets, as well as imbibing serum and will become covered by a canopy of endothelium.
2. Necrosis will occur in the avascular intima around the fibrin.
3. The intima then receives a vascular network providing the opportunity for hemorrhage and the deposit of serum and formed elements that are rich in lipid.

*Atheroma - Greek for porridge or gruel.

4. The stretch and recoil of the artery after each heartbeat pulls at the organizing thrombus perseveringly and causes microscopic disruptions of fibrous attachments which bleed.

5. Finally, should arterial tissues be stretched beyond their elastic limits at stress points as was proposed to happen at the ostia of the intercostal arteries, tissue thromboplastin would escape predisposing to fibrin formation. Also intimal necrosis and fibrin tearing would allow the escape of additional thromboplastin encouraging repeated fibrin deposition.

Part C -- Intimal Filtration of Lipid in the Pathogenesis of Atherosclerosis.

In this hypothesis the primary event is thought to be a depositing of lipid that is related to local trauma and metabolism. The approach will now shift to investigations of microscopic anatomy and physiological functions in the intima as a solution to atherogenesis. A good place to begin would be a report[50] of 50 autopsies where only one aorta provided a fibrin thrombus and where the most common alteration was fatty metamorphasis. The natural history of atherosclerosis begins in childhood. The earliest alteration is the fatty streak, which is a pin-sized, yellow deposit as seen grossly or a large aggregation of lipid-filled macrophages or foam cells as seen microscopically. Geiringer[51] of the other school feels the earliest atherosclerosis

begins is eight years (as a fibrin deposit); however, Holman and McGill (1958)[1] after 526 autopsies in New Orleans claim foam cells in every case from three years or older regardless[52] of race, sex, diet, blood pressure, serum lipid level, or other known environmental or genetic factors. The percent of surface area involved by fatty streaks rises slowly until eight years of age when the extent of the lesions increases precipitously in the Negro. In whites a precipitous spread of foam cell deposits happens at puberty and does not peak as high as in the Negro. Fibrous plaques begin in the second decade but do not increase appreciably until the fourth decade, roughly paralleling the prior development of fatty streaks but lagging by 15 years. The severity of the condition at 40 years of age (the age limit of the series) was highest in the white male and consistently lowest in white females; the Negro of both sexes fell between.

Restrepo and McGill[53] compared 289 autopsies in Cali, Columbia, and Strong and McGill[54] compared 226 and 113 autopsies in Guatemala and Costa Rica respectively with the New Orleans group. The findings showed fatty streaks occurred in the three groups at the same rate and age but fibrous plaques were much less extensive among Central Americans, who consume much less fat and protein than the people of New Orleans, having minimally adequate diets high in carbohydrate. Numerically more severe complications, such as ulceration, etc., were in the U. S. A. group. It was

stated that the wide individual variations prevented statistical significance to the 1% level but the authors declared that the general trend was valid.

Analysis of the lipid accumulations[55, 56] (see Table 2) finds cholesterol as the principle lipid fraction with the ester form more plentiful than the free form. Bottcher relates he encountered wide differences in lipid content, but the coronary and cerebral arteries consistently had larger quantities of lipid than did the aorta, even when normal. The former arteries also took on lipid five times over the normal, while the aorta only gained three to eleven percent more lipid. Hirsch[57] approximated Table 2 in his analyses.

In situ synthesis of cholesterol[58, 59, 60, 61, 12] has been detected in rabbit, calf, and rat aortas and no doubt occurs in humans but not at rates conducive to atheroma formation. Atheromatous degeneration has been induced in laboratory animals on a wholesale scale for years simply by giving their standard diets a cholesterol suppliment. The object has been to gain insight into the manner by which oral cholesterol is transformed in a matter of weeks into a state in quadrapeds that is remarkably similar to clinical disease. Most experts agree that the cholesterol deposited comes from an extrinsic source, namely the blood. However, the thrombotic encrustation school debates thrombus and recurrent hemorrhage as the mediators of lipid from

PERCENT COMPOSITION OF LIPID

		Aorta		Coronary		Circle of Willis	
		O/I	II/III	O/I	II/III	O/I	II/III
1.	phospholipid	58	33.5	28.4	32.1	58.2	37.5
2.	ffa	8.0	2.0	6.7	4.9	5.7	4.1
3.	free cholesterol	10.1	19.4	6.1	11.9	9.3	14.7
4.	cholesterol esters	8.8	36.1	8.8	24.6	7.5	28.1
5.	triglycerides	15.0	10.6	49.7	26.6	20.0	16.0
6.	average age (yrs.)	18	57	20	47	48	68

Stage 0 - No lesion at 10x magnification
Stage I - Fatty streaks or spots
Stage II - Fibrous plaques and/or atheromas
Stage III - Lesions as above with additional ulceration, etc.

Table 2

COMPARISON OF LIPID COMPOSITION
OF BLOOD PLASMA AND ARTERIAL TISSUE*

		Plasma	Intima	Early Plaques	Media
1.	free cholesterol	14.1	14.2	16.2	17.3
2.	ester cholesterol	38.3	38.6	38.5	16.7
3.	phospholipid	22.8	20.1	19.0	34.1
4.	neutral fat, etc.	23.3	27.1	26.3	31.9

*percentages of total lipid.

Table 3

the serum permeates the endothelium and is "filtered off"[9, 10] against the internal elastic membrane.

Page and Kirk (1943)[62] published a comparison of lipid in the blood and arterial tissue (see Table 3) and concluded that plasma permeates the intima. The fatty acids of blood and plaques are nearly identical in quantity and quality[63] as are the lipoproteins Sf 12-100.[64] Cholesterol isotope was given intraperitoneally to mice[65] and found to enter the intima by three hours and was at greatest concentration by 20 hours; after that counts in the media surpassed the intima leading the observer to the conclusion that the lipid, which perfused the intima in the first hours, then gathered in the media, but no estimation of the extent intimal perfusion or the role vasa vasorum played in contributing lipid could be made. Duncan[66, 67, 68] injected labeled albumin (RISA, Abbott) and fed cholesterol to dogs; he reported the isotopes diffuse into the aorta from the lumen at progressively faster rates along the vessel as points nearer the heart are chosen. The gradient of transfer corresponded to changes in circumferential tension. (Higher perfusion pressure in vitro can enlarge the collection of aortic cholesterol.[69]) The amount of isotope distally steadily enlarged and came to equal amounts in proximal areas obliterating the former gradient if the experiment ran above 130 days. Duguid believed that the gradient in cholesterol uptake was no different after 130 days, but that the

lipid was dissipated from proximal walls faster than it was distally causing a pile up. C^3H labeled cholesterol in rabbits[70] accumulates at an exponential rate with intima/plasma ratios enlarging from 0.39 at 21 days to 1.00 at 87 days indicating the material was transported into the area and not synthesized *in situ*. The influx of cholesterol (rats) was significantly higher than net accumulations by factors of five to ten; this large turnover in and out was nine to ten times greater on a 1% cholesterol supplement than in comparable animals on a 0.2% diet: these data suggest that the amounts of cholesterol eaten is a factor in determining the amount deposited.

A comparison of lipid uptake in small and large-sized dogs proved that the gradient was determined by the location along the aorta and not by actual wall thickness.[71] Tangential tension across the vessel wall might break endothelial bonds creating "pores" and expose the intima to serum (Harman 1962).[10] The arch of the aorta and other points of stress would be more permeable on this basis. Vessel distension in hypertensive rats[72] caused intimas to fill with rbc's, macrophages, leukocytes, rbc fragments, and serum and caused endothelial cell degeneration. Pathologists have offered light and electronmicroscope evidence for and against the existence of pores.[73, 6, 7, 74, 75, 76, 77, 78] Curiously enough, antibody techniques[79] detected only one serum protein of any concentration in human aortas, whether normal or

degenerate; that blood constituent is beta lipoprotein. *Vide supra* Albumin (RISA)[68] did enter the intima but four times faster than cholesterol; also[70] free cholesterol was better able to penetrate the intima than its esterified form by a factor of two to four. The former author was convinced that the two isotopes rode into the intima attached to beta lipoprotein. Stewart[80, 81] *in vitro* pulsed normal and hyperlipemic serums at physiological pressures against the walls of freshly autopsied rabbit aortas and produced subintimal lipid deposits successfully only by using low density lipoprotein from cholesterol-fed rabbits; neither alimentary lipemia (chylomycrons from the thoracic duct), triglycerides, or lipoprotein of higher Sf values would enter. The addition of fibrinogen to the low density lipoprotein enhanced the size of the deposit as well as its depth. The idea of fractionalization and apparent active uptake of serum contents is not easy to understand, if serum penetrates the endothelium by simple filtration or diffusion through pores. Consideration must be given to an alternate or a second possibility, namely metabolic work performed by the endothelial cells or subendothelial tissues.

It seems fitting to include one paragraph on an important substrate - beta lipoprotein. Of the plasma protein 12 to 15% is lipoprotein;[4] however, 70% of normal fasting serum lipid is in the beta fraction. Ultracentrifuge technique has found the molecular

species vary in rate of flotation from two to 40,000 Svedberg units with the beta group in the range of 25-40 Sf units. Low density lipoproteins are so designated with Sf values below 400. According to Page lipoprotein from the blood represents lipid that has escaped from the metabolic machinery of the hepatic parenchyma where it was participating in metabolism. Lipoproteins with Sf values above 70 units contain more neutral fat and less cholesterol than lower members who are often called cholesterol rich. The beta fraction rises in men with aging[4] suggesting that they have a greater ability to deposit cholesterol. The liver[82] may well be the master regulator of serum lipid levels and be responsible, in part, for atherosclerosis. Yet in a comparison of aortic cholesterol content and atherosclerosis with serum concentrations of lipid, Lande (1936)[83] in 123 healthy people, who died by violence, could find no correlation. Also Faber (1946)[84] could find no correlation. Paterson (1960)[85] followed some 800 ambulatory patients confined to institutional living from 1953 to 1959; examination of 191 fatalities drew out little correlation except "perhaps" with levels above 300 mg.%. Gofman claims the Sf 10-20 class of lipoprotein to be definitely associated with atherosclerosis and reports consistent elevation in patients with myocardial infarction, angina pectoris, diabetes, myxedema, nephrosis, and xanthoma tuberosum. He suggests that the severity of intimal deposits depends, in part, on the concentration of serum lipoproteins

class Sf 10-20. He further believes abnormal levels of the Sf 10-20 class represents a disorder of general metabolic handling of fats.[86, 87]

Electronmicroscope sections (1962) of human and rabbit plaques picture the endothelial cells interdigited but the aortic lumen is sometimes separated from the intima by cell processes reduced to the width of two cytoplasmic membranes, about 100 to 200 angstroms wide. Beneath the endothelium lies a "deep margin" of extracellular space before the underlying cells of the lesion are met. Intracellular lipid is present in three visible forms:[75] In early fatty streaks smooth muscle cells contain a fine network of lipid inclusions definitely inside a double-layered membrane; they also have a second prominent form of lipid that is dense, homogenous, and cytoplasmic without a limiting membrane. Foam cells occasionally sported similar inclusions, but most often their lipid is present as clear vacuoles. Extracellular lipid is seldom seen. In the more grossly visible plaque the same elements remain that were in the fatty streak, but in different proportions. Lipid is seen in the two varieties of cell in its cytoplasmic form mainly without a limiting membrane. Smooth muscle cells are so loaded with fat that they resemble foam cells and are proposed by the author to be just that. Heavily laden cells become degenerate. In addition,

these areas contain fibrocytes, white fibrous and yellow elastic fibers, and numerous extracellular fat globules.

The origin of foam cells is interesting. In 1941 Leary[77] postulated that macrophages migrate from the liver already filled with lipid to enter the intima. Their specific gravity along with hydrostatic pressure were what caused them to stay near the vessel wall. Leary's last publication[88] to support this idea was 1951.* Also in 1951 macrophages carrying isotope were recirculated in the blood and did circulate freely without ever coming to rest inside atheroma formations.[78] McMillan[89] reported mitoses in foam cells then suggesting that they arise in situ. Rebuck and Crowly[90] published the skin window technique in 1955 whereby inflammatory cells can be examined on permanent slides in multiple time intervals after a skin wound has been freshly made. Lymphocytes, comprising 50 to 70% of a normal differential count, are capable of intravascular ameboid action and phagocytosis; Rebuck demonstrated lymphocytes migrate into an inflammed area and transform into the macrophages, which actively ingest debris. Neutrophils arrive first as an initial response to injury, phagocytize, and release antibacterial agents from the cytoplasmic granules.[91] Macrophages occupy an increasingly larger percentage of the two cell population in a wound as the hours pass so that slide counts pass the 50% point at nine to twelve hours to reach

*Cited by Campbell Moses[54]

70 to 95% macrophages by one to two days. Later the macrophage is proposed to further transform into a fibrocyte, passing through the clasmocyte stage, and thus not only supply cells for debridement and antibacterial action but also for repair. In atherosclerosis the foam cells reposited in the lesion possibly may have several origins such as the phagocytizing smooth muscle cells (see above); however an important source for these cells would seem to be the vascular lymphocyte.

The idea of metabolic work is supported by the appearance of endothelial cells. Above plaque areas these cells are swollen with hypertrophy of the golgi apparatus and endoplasmic reticulum.[92, 73] Human endothelial cells in tissue culture will ingest cholesterol and beta lipoprotein until visibly enlarged, and will obligingly have returned the materials to the outside about five days after the media is changed to normal.[93] Phagocytosis[74] and pinocytosis[73] are probably responsible for these cell's similar ingestion of thorium dioxide granules in vivo.[92] Lipid never accumulates inside but is extruded subendothelially where foam cells, very metabolically active in this location,[5] phagocytize eagerly even after heavily laden with visible fat.[92]

C. S. Lewis in 1924 had explained the wheal and flare of his "triple response" as due to an inflammatory mediator - histamine. His work had been corraborated years ago. Histamine affects upon endothelial cells[8] the ability in vivo to attack India ink

particles, which adhere for a time, and over 24 hours are ingested and eventually are passed into the cellular regions outside the lumen. Antihistaminic drugs minimize this response.[8] Mechanical trauma causes the same response as applied histamine except last longer. Histamine effects upon the vascular tree had been shown by Majno in 1961[6, 7] to open endothelial "pores" 0.1 to 0.8 mm., which permitted immediate tissue edema and vascular stasis. However, only the venule side had experienced the action in vessels sized from seven microns to near 80 microns; maxium effect had occurred in sizes 20 to 30 microns. Majno had clear photomicrographs of the arterioles and capillaries in the areas tested with histamine completely intact while venules alongside were leaking profusely. Schayer[94] had followed histadine decarboxylase concentrations in many tissues and proposed an interesting concept: histamine is present in two forms, which are called "bound" and "induced." The bound variety is histamine stored in mast cells and released rapidly when this cell is exposed to substrate from damaged cells. The bound histamine initiates a rapid reply of maximum degree, i.e., the triple response or more generalized inflammation. Antihistamine can antagonize this early response to some extent. The induced histamine is the proposed product of viable cells synthesized because they are bathed by damaged cell substrates or are injured themselves. The induced histamine maintains the chronic inflammation

needed for healing until such a time as mending is completed. A steady release of this potent hormone in minute quantities out of reach of tissue inhibitors and drugs can slowly develop a tissue reaction after a latent period that is of long duration; it cannot be accurately simulated by outside injection. Schayer commented that the best evidence in 1961 suggests its formation does occur in or near vascular endothelial cells.

Mechanical injury in the arterial system was shown to be a possibility in an earlier section of this paper. Damaged endothelial cells and subendothelial tissues might synthesize induced histamine during their repair. The stimulated endothelial cells would then become rolled up, have hypertrophied organelles, and begin to phagocytize serum lipoprotein. Stress areas along the arterial tree where cellular damage is common place, might regularly be pouring large amounts of lipoprotein rich in lipid into the transportation system of the wall as a secondary effect of their repair reaction. Harman[9, 10] proved that an antihistamine like chlorpheniramine can restrain experimental atherosclerosis but not eliminate it. Dury[11] placed cholesterol-fed rabbits on two weeks of cortisone therapy and found a marked regression or complete absence of aortic degeneration. Their evidence seems to point to injury and inflammation as major participants in atherosclerosis.

Cholesterol and its esters cause a chronic granuloma where they are injected subcutaneously. Grossly large lesions appear, and

microscopic inspection of these finds heavy inflammation with many macrophages, giant cells as well as considerable fibroblastic proliferation.[95] Adams[96] established that C_3H labeled cholesterol injected subcutaneously into rats is mainly found extracellularly as a crystal in the lesion. When mixed with the phospholipids syringomyelin and lecthin, the cholesterol will appear almost entirely inside foam cells with few signs of chronic granuloma formation in the site of injection. The conclusions he drew were: cholesterol has a sclerosing effect and phospholipid can attenuate this effect by making the material accessible to phagocytosis and transport.

Earlier low density lipoprotein with attached albumin and cholesterol was proposed to permeate the intima, passing from the lumen in a steady turnover, Page and Kirk,[73] and Duncan.[66, 67, 68] The lipid precipitated in the intima was thought only a fraction of the amount actually transported across this tissue.[70] Harman[97] postulated that the key to the initial separating out of small fractions of lipid is a process of oxidative polymerization of the low density serum lipoprotein. The process would be a simple polymerization with oxygen like the drying of paint; the yield would be higher molecular weight lipoperoxides and their secondary products. Antioxidants like ascorbic acid, thiamine, iodide, vitamine A palmitate, and alpha-tocopherol have lessened atherosclerosis in rabbits, possibly on the above basis.

Lipoperoxides are unstable and reactive; they may "anchor" themselves chemically to tissue protein doing cellular damage and perpetuating the release of induced histamine. Out of lipoperoxide disintegration would come cholesterol, fatty acids, calcium, and other materials that are not only irritating but also hydrophobic; these will then "filter off" against the internal elastic membrane as an intimal precipitate.

The vessel wall evidently has some defense against the depositing of lipid because, as was noted earlier, forty human years or more are needed before intracellular lipid (foam cells) becomes an extracellular, crystallized, and calcified mass with a thick tissue halo of fibrosis. Adams[5] believes the phospholipid syringmyelin is produced in the media as a dispersing agent. He found that phospholipid is present only in the elastic lamellae of normal medias, but its formation is accelerated in atherosclerosis creating extracellular pools in the intima. Staining for cellular enzymes like lactic dehydrogenase, he found cellular metabolism is uniform across the normal vessel wall as would be expected. But in areas of degeneration focal elevations in enzyme activity appear in the subendothelial macrophages from adolescence onwards. The enzymes of the middle 1/3 of the aortic media deep to the involved areas become decreased in early adulthood often progressing to complete absence by the time the process is advanced. He feels the enzyme loss represents a

progressive shutdown of the metabolic machinery producing phospholipid. He thinks the shutdown is due to ischemia, but Winternitz and Geiringer vide supra demonstrated the complex secondary vascularization that occurs in response to intimal thickening. Cholesterol, however, antagonizes the action of phospholipid on emulsions and seems to interfere with its synthesis.[98] Chemical analysis of intimas finds the phospholipid cholesterol ratio (PC ratio) in rabbit atheromas falling from 4.7 at two weeks to 0.68 after 12 weeks. In humans the ratio is greater than one in early lesions and falls below unity as the condition advances. Phospholipid does rise sharply in concentration early in the disease, but cholesterol rises outstrip it later. Despite a possible tangle in thinking about reasons for enzyme losses, the facts seem to be: 1. phospholipid attenuates the effects of cholesterol on tissue, 2. phospholipid in plaque areas appears in sizeable amounts, 3. phospholipid synthesis does occur in the vessel wall at rates useful to this schema,[99, 100, 101, 1
4. many years must pass with nothing but foam cell deposits before lipid becomes extracellular in the human aorta.

In rabbits on cholesterol diets, not a trace of enzyme leaves the media even if the atheromas are florid; Adams explains rabbit atherosclerosis on the basis of simple overloading of the transportation system so that while phospholipid production does not seem diminished, it cannot solubilize the overload of lipid

rich lipoprotein. He feels the rabbit precipitates form as they do in the human with identical effect but for a different reason.

In summary, the filtrationist ideas in stepwise fashion are:

1. At stress points along the artery, endothelial cells and subendothelial tissues are regularly damaged and a release of induced and bound histamine occurs during their repair.
2. The histamine in turn stimulates the endothelial cells to collect serum lipoprotein by phagocytoses and pinocytosis and to deposit it in the intima.
3. Vascular lymphocytes migrate to the intima, transform into macrophages, and avidly ingest any lipid or debris deposited there; an alternate idea that is also popular points to phagocytizing smooth muscle cells as the source of foam cells.
4. The turnover of serum lipoprotein is large, yet only a fraction becomes deposited in the intima; oxidation of small amounts of lipoprotein into lipoperoxides plants into the tissue a reactive chemical that does cell injury and perpetuates the release of histamine.
5. Cholesterol, fatty acids, calcium, and other materials released from degenerate lipoprotein have a hydrophobic nature which tends to cause their filtering off against the internal elastic membrane as intimal precipitates.

6. Phospholipid is synthesized in the media to disperse and to solubilize the precipitates so that they can be phagocytized or transported away.
7. In human aortas phospholipid synthesis falls off as foam cell deposits thicken with the result that lipid deposition is accelerated.
8. The irritating properties of lipid breakdown products enhance histamine release and stimulate heavy fibrosis.
9. In rabbits no medial necrosis occurs but the PC ratio falls remarkably. Probably phospholipid synthesis remains intact but its production as well as lipid transport cannot keep up with the overload fast enough to prevent falling out.
10. Chlorpheniramine and cortisone reduce rabbit atheroma probably by cutting back injury-accentuated lipoprotein uptake.

CONCLUSION

Atherogenesis was discussed in three sections.

Part A, Endothelial, intimal, or medial injury from arterial distention and recoil in rhythmic cadence with the heartbeat for a lifetime was considered to be an important factor accepted by many researchers; ideas vary, and there were several possible ways presented where repeated, arterial movement might be linked with lipid deposition.

Two divergent approaches to the subject were outlined:

Part B, "Thrombotic encrustation" described a school of thought centered around the formation and subendothelial incorporation of fibrin thrombi, which were proposed to act as a nidus for fibrosis and cholesterol accumulation. This hypothesis stated that microscopically-sized fibrin forms on arterial walls either spontaneously or where there is underlying wall disease; the fibrin then would imbibe serum and attach wbc's, rbc's, and platelets before being covered by endothelium. Isolated in the intima the fibrin would disintegrate to release its trapped lipids and to cause in time vascularization with periodical bleeding as the vessel is stretched by each pulse wave. The end result would be an atherosclerotic plaque of fibrosis and extracellular lipid, which in some way is mainly cholesterol and not a mixture of other blood lipids. Part C, "Filtration" is the key to the second approach whereby very small fractions of the lipoprotein that permeate the intima from the serum was supported to become insoluble and to filter off against the internal elastic membrane. Held responsible in this hypothesis was a minor chemical reaction slowed far to the left but proposed to occur when oxygen contacts lipoprotein, namely, polymerization. High molecular weight molecules of aggregated lipoprotein then would become trapped and later disintegrate in the intima.

The "filtration" hypothesis seems more feasible to this student of the subject for two reasons: 1. evidence was presented

to show that only very selective fractions of serum protein filters through the intima, i.e., the only protein appearing in any concentration in the human aorta is beta lipoprotein (Sf 25-40), which contains 70% of the normal fasting serum lipid and is often called cholesterol rich. 2. Endothelial cells change some in their morphology and phagocytize serum contents *in vivo* andebeta lipoprotein *in vitro* under histamine stimulation. Subendothelial degeneration and/or vessel injury from stretching beyond the vessel's elastic limits, as might occur at intercostal arteries, could cause induced histamine production and chronic inflammation. The predominance of cholesterol in the atherosclerotic plaques, rather than a mixture of lipids, and the effects produced in endothelial cells by histamine both point to some metabolic regulation of intimal degeneration. They thereby point more to the "filtration" hypothesis as it is constructed today and to inflammation as a plausible factor than any pieces of evidence presented by the other hypothesis. According to the "thrombotic encrustation" hypothesis lipid composition would be expected to be less uniform or patterned than it is with more diffuse involvement of vessel walls instead of discrete plaques.

SUMMARY

Allowing the assumption that the inflammatory capability in the human aorta is significantly paralleled by that in the skin, a study of the response in the skin to a standard skin abrasion was

done; a group of 19 men who had suffered clinically documented myocardial infarction at a relatively young age (35 to 59 years) volunteered along with a group of comparable controls. The percentages of macrophages or secondary invaders were determined nine hours after the abrasion was made. The heart attach group averaged 39.0 ± 19 while the controls averaged 25.4 ± 11; the P value was less than 0.01. It is respectfully suggested that a standard skin abrasion after more extensive testing and refinement might be suitable as a clinical test.

BIBLIOGRAPHY

1. Holman, R. L. and others, Natural History of Atherosclerosis: Early Aortic Lesions as Seen in New Orleans in the Middle of the 20th Century, Am. J. Path. 34:209-236, 1958.

2. Duguid, J. B. and Robertson, W. B., Mechanical Factors in Atherosclerosis, Lancet 1:1205-1209, 1957.

3. Astrup, T. C., Role of Blood Coagulation and Fibrinolysis in the Pathogenesis of Arteriosclerosis. (In: Page, I. H., ed., Connective Tissue, Thrombosis and Atherosclerosis, New York, Academic Press, 1959, p. 223)

4. Page, I. H., Atherosclerosis: An Introduction, Circulation 10:1-27, 1954.

5. Adams, C. W. and others, The Distribution of Lipids and Enzymes in the Aortic Wall in Dietary Rabbit Atheroma and Human Atherosclerosis, J. Path. Bact.86:421-430 (Oct.) 1963.

6. Majno, G. E. and Palade, G. E., Studies on Inflammation: I Effect of Histamine and Serotonin on Vascular Permeability; Electronmicroscope Study, J. Biophys. & Biochem. Cytol. 11:571-606, 1961.

7. Majno, G. E. and others, Studies on Inflammation: II The Site of Action of Histamine and Serotonin Along the Vascular Tree: A Topographical Study, J. Biophys. & Biochem. Cytol. 11:571-626, 1961.

8. Matoltsy, Gedeon A. and Matoltsy Margit, Action of Histamine and Antihistaminic Substances on the Endothelial Cells of the Small Capillaries in the Skin, J. Pharmacol. & Exper. Therap. 102:237-249, 1951.

9. Harman, Denham, Atherosclerosis: Inhibiting Effect of Chlorpheniramine, Circulation Research 8:184, 1960.

10. _____, Atherosclerosis: Inhibiting Effect of an Antihistaminic Drug, Chlorpheniramine, Circulation Research 11:277-282 (Aug.) 1962.

11. Dury, A. J., Lipid Metabolic Alterations Associated with Cortisone - Induced Regression of Cholesterol Atherosclerosis in the Rabbit, Fed. Proc. 15:52-62, 1956.

12. Rebuck, J. W. and Crowley, J. H., Method of Studying Leukocytic Functions in vivo, Ann. New York Acad. Sc. 59:757-805, 1955.

13. Texon, M. L., A Hemodynamic Concept of Atherosclerosis, with Particular Reference to Coronary Occlusion, Arch. Int. Med. 99:418-427, 1957.

14. Willis, G. C., Localizing Factors in Atherosclerosis, Canad. Med. Ass. J. 70:1-20, 1954.

15. Duguid, J. B., Atheroma of the Aorta, J. Path. & Bact. 29:371-387, 1926.

16. Harrison, C. V., Experimental Arterial Disease Produced by Cholesterol and Vitamin D, J. Path. & Bact. 36:447-453, 1933.

17. Hicham, John B., Pulmonary Hypertension. (In: Cecil-Loeb, Textbook of Medicine, 11th ed., Philadelphia & London, W. B. Saunders Co., 1963, p. 569-571)

18. Duguid, J. B., Thrombosis as a Factor in the Pathogenesis of Coronary Atherosclerosis, J. Path. & Bact. 58:207-219, 1946.

19. _____, Thrombosis as a Factor in the Pathogenesis of Aortic Atherosclerosis, J. Path. & Bact. 60:57-62, 1948.

20. _____, Pathogenesis of Atherosclerosis, Lancet 2:924-927, 1949.

21. Taylor, C. B. and others, Localized Atherosclerotic Lesions Induced in the Aorta of the Juvenile Rabbit by Freezing, Arch. Path. 49:623-636, 1950.

22. Levene, C. I., The Electronmicroscopy of Atheroma, Lancet 2: 1215-1630, 1955.

23. Harrison, C. V., Experimental Pulmonary Atherosclerosis, J. Path. & Bact. 60:289-293, 1948.

24. Heard, B. E., An Experimental Study of Thickening of the Pulmonary Arteries of Rabbits Produced by the Organization of Fibrin, J. Path. & Bact. 64:13-28, 1952.

25. McLetchie, N. G. B., Pathogenesis of Atheroma, Amer. J. Path. 28:413-436, 1952.

26. Crawford, T. E. and Levene, C. I., The Incorporation of Fibrin in the Aortic Intima, J. Path. & Bact. 64:523-531, 1952.

27. Geiringer, E. F., Intimal Vascularization in Atherosclerosis, J. Path. & Bact. 63:201-211, 1951.

28. Winternitz, M. C. and others, The Biology of Atherosclerosis, Springfield, Charles C. Thomas, 1938, p. 22. Cited by: Moses, Campbell, Atherosclerosis, Mechanisms as a Guide to Prevention, Philadelphia, Lea & Febiger, 1963, Chapter II.

29. Paterson, J. C., Capillary Rupture with Intimal Hemorrhage as a Causative Factor in Coronary Thrombosis, Arch. Path. 25:474-490, 1938.

30. Morgan, A. D., The Pathogenesis of Coronary Occlusion, Springfield, Charles C. Thomas, Oxford Press, 1956.

31. Moses, Campbell, Atherosclerosis, Mechanisms as a Guide to Prevention, Philadelphia, Lea & Febiger, 1963, Chapters II and III.

32. Paterson, J. C., Capillary Rupture with Intimal Hemorrhage as a Cause of Pulmonary Hemorrhage, Am. Heart J. 18:451-470, 1938.

33. Paterson, J. C. and others, Hemosiderin in Early Atherosclerosis Plaques, Arch. Path. 61:496-515, 1956.

34. _____, The Serum Lipids in Human Atherosclerosis, An Interim Report, Circulation 13:224-229, 1956.

35. _____, The Gofman Indices of Coronary Atherosclerosis, Canad. Med. Ass. J. 74:538-542, 1956.

36. Paterson, J. C., Vascularization of Early Atherosclerotic Plaques, Arch. Path. 64:129-135, 1957.

37. Paterson, J. C. and others, The Mechanism of Progression of Atherosclerotic Plaques, Conn. Med. 25:93-100, 1961.

38. Paterson, J. C., Heart Disease and Employment. Stress, Intimal Hemorrhage and Coronary Occlusion, J. Occup. Med. 3:59-69, 1961.

39. Chandler, A. B. and Hand, R. A., Phagocytized Platelets: A Source of Lipids in Human Thrombotic and Atherosclerotic Plaques, Science 134:946-949, 1961.

40. Willis, G. C., The Frequency, Pathogenesis and Significance of Intimal Hemorrhage, Canad. Med. Ass. J. 67:644-650, 1952.

41. More, R. H. and others, Role of Mural Fibrin Thrombi of the Aorta in the Genesis of Atherosclerotic Plaques; Report of Two Cases, Arch. Path. 63:612-616, 1957.

42. Movat, H. Z. and others, The Morphologic Elements in Early Lesions of Atherosclerosis, Am. J. Path. 35:93-97, 1959.

43. Haust, M. D. and others, The Mechanism of Fibrosis in Arteriolosclerosis, Am. J. Path. 35:265-270, 1959.

44. Haust, M. D., The Role of Smooth Muscle Cells in the Fibrinogenesis of Arteriolosclerosis, Am. J. Path. 37:377-383, 1960.

45. Horn, H. B. and Finkelstein, L. E., Atherosclerosis of the Coronary Arteries with Special Attention to Fibrinoid Lesions, Arch. Path. 22:183-205, 1936.

46. Heard, B. E., Mural Thrombosis in the Renal Artery and its Relation to Atherosclerosis, J. Path. & Bact. 61:635-642, 1949.

47. Mole, R. H., Fibrinolysin and the Fluidity of the Blood Post Mortem, J. Path. & Bact. 60:413-420, 1948.

48. Astrup, T. C., The Biological Significance of Fibrinolysis, Lancet 2:565-577, 1956.

49. Todd, A. S., Fibrinolysis Autographs, Nature 181:495-500, 1958.

50. Wissler, R. W. and others, A Study of the Histogenesis of Atherosclerosis in Man, Circulation 18:497-511, 1958.

51. Geiringer, E. F., The Gerontological Aspects of Atheroma, An Approach to the Pathology of Senescence, Brit. J. Soc. Med. 2:132-138, 1948.

52. Holman, R. L. and others, The Arterial Wall as an Organ, Circulation 16:483-490, 1957.

53. Restrepo, C. L. and McGill, H. C., Early Lesions of Aortic Atherosclerosis in Cali, Colombia, A.M.A. Arch. Path. 67:618-623, 1959.

54. Strong, J. and others, The Natural History of Atherosclerosis. Comparison of the Early Aortic Lesions in New Orleans, Guatemala, and Costa Rica, Am. J. Path. 34:731-744, 1958.

55. Gofman, J. W. and others, The Role of Lipids and Lipoproteins in Atherosclerosis, Science 111:166-171, 1950.

56. Bottcher, C. J., Lipid and Fatty Acid Composition of Coronary and Cerebral Arteries at Different Stages of Atherosclerosis, Lancet 2:1163-1166, 1960.

57. Hirsch, Edwin, An Analysis of the Causal Factors of Atherosclerosis, A.M.A. Arch. Int. Med. 102:1024-1035, 1958.

58. Feller, D. D. and Huff, R. L., Lipid Synthesis by Arterial and Liver Tissue Obtained from Cholesterol-Fed and Cholesterol Alcohol-Fed Rabbits, Am. J. Physiol. 182: 237-241, 1955.

59. Chernick, S. A. and others, The Metabolism of Arterial Tissue II. Lipide Synthesis: The Formation in vitro of Fatty Acids and Phospholipids by Rat Artery with C^{14} and P^{32} as Indicators, J. Biol. Chem. 179:113-124, 1947.

60. McCandless, E. L. and Zilversmit, D. B., Aortic Synthesis of Plaque Phospholipid in Eviscerated, Nephrectomized Cholesterol-Fed Rabbit, Abst., Circulation 16:483-487, 1957.

61. Werthessen, N. T. and others, Factors Influencing the Biosynthesis of Lipids by Calf Aorta in vitro, presented at the American Society for Study of Arteriosclerosis, Ninth Annual Meeting, (Nov. 5, 6) 1955. Cited by: Moses, Campbell, Atherosclerosis, Mechanisms as a Guide to Prevention, Philadelphia, Lea & Febiger, 1963, Chapter II.

62. Hirsch, E. F. and Weinhouse, S. M., The Role of the Lipids in Atherosclerosis, Physiol. Rev. 23:185-202, 1943. Cited by: Hirsch, E. F., An Analysis of the Causal Factors of Atherosclerosis, A.M.A. Arch. Int. Med. 102:1024-1035, 1958.

63. Tuna, N. I. and others, The Fatty Acids of Total Lipids and Cholesterol Esters from Normal Plasma and Atheromatous Plaques, J. Clin. Invest. 37:1153-1159, 1958.

64. Hanig, M. S. and others, Flotational Lipoproteins Extracted from Human Atherosclerotic Aortas, Science [illegible]:176-185, 1956.

65. Adams, C. W. and others, A Hypothesis to Explain the Accumulation of Cholesterol in Atherosclerosis, Lancet 1:890-892, 1962.

66. Duncan, L. E., Circulation of Labeled Albumin Through the Aortic Wall of the Dog, Circulation Research 7:390-397, 1959.

67. Duncan, L. E. and Buck, Kathryn, Passage of Labeled Cholesterol into the Aortic Wall of the Normal Dog, Circulation Research 7:765-770, 1959.

68. _____, Quantitative Analysis of the Development of Experimental Atherosclerosis in the Dog, Circulation Research 8:1023-1027, 1960.

69. Werthessen, N. T., Perfusate Pressure as Related to Cholesterol Deposition Within the Surviving Aorta, Fed. Proc. 13:163, 1954.

70. Newman, H. C. and Zilversmit, D. B., Quantitative Aspects of Cholesterol Flux in Rabbit Atheromatous Lesions, J. Biol. Chem. 237:2078-2084, 1962.

71. Duncan, L. E. and Buck, Kathryn, Comparison of Rates at Which Albumin Enters Walls of Small and Large Aortas, Amer. J. Physiol. 203:1167-1172, 1962.

72. Esterly, J. A. and others, Altered Permeability of the Renal Artery of the Hypertensive Rat: An Electronmicroscope Study, Amer. J. Path. 43:619-638 (Oct.) 1963.

73. Buck, R. C., Fine Structure of Aortic Endothelial Lesions in Experimental Cholesterol Atherosclerosis of Rabbits, Amer. J. Path. 34:897-910, 1958.

74. Lautsch, E. V. and others, Surface Studies of the Early Development of Cholesterol Atherosclerosis in the Rabbit, Circulation 6:464, 1952.

75. Geer, J. C., Fine Structure of Human Atherosclerotic Lesions, Amer. J. Path. 38:263-288, 1961.

76. Poole, J. C. F. and Florey, H. W., Changes in the Endothelium of the Aorta and the Behavior of Macrophages in Experimental Atheromata of Rabbits, J. Path. & Bact. 75:245-252, 1958.

77. Gordon, I. G., Mechanism of Lipophage Deposition in Atherosclerosis, Arch. Path. 44:247-253, 1947.

78. Simonton, J. H. and Gofman, J. W., Macrophage Migration in Experimental Atherosclerosis, Circulation 4:557-601, 1951.

79. Tracy, R. E. and others, On the Antigenic Identity of Human Serum Beta and Alpha-2 Lipoproteins and Their Identification in the Aortic Intima, Circulation Research 9:472-478, 1961.

80. Stewart, G. T., The Production of Intimal Deposits of Lipoid in Isolated Rabbit's Aorta, Brit. J. Exp. Path. 4: 389-394, 1960.

81. _____, Pulsation of Lipids, Lipoprotein and Fibrinogen Against Excised Segments of Rabbits' Aortas, Brit. J. Exp. Path. 43:345-349, 1962.

82. Ashworth, C. T. and others, Hepatic Lipids, A.M.A. Arch. Path. 72:620-624, 1961.

83. Lande, C. E., Human Atherosclerosis in Relation to Cholesterol Content of the Blood Serum, Arch. Path. 22:301-307, 1936.

84. Faber, Mogens, The Cholesterol Content of the Human Aorta in Relation to the Serum Cholesterol Concentration, Acta. Med. Scand. 125:418-421, 1946.

85. Paterson, J. C., Serum Cholesterol Levels in Human Atherosclerosis, Canad. Med. Ass. J. 82:6-16, 1960.

86. Gofman, John and others, Lipoproteins and Atherosclerosis, J. of Gerontology 6:105-119, 1951.

87. _____, Lipoproteins, Coronary Heart Disease, and Atherosclerosis, Physiol. Rev. 34:589-607, 1954.

88. Leary, T. M., Cholesterol and Man, J. of Gerontology 6: (Supp. to No. 3) 118-121, 1951.

89. McMillan, G. C. and Duff G. L., Mitotic Activity in the Aortic Lesions of Experimental Cholesterol Atherosclerosis in Rabbits, Arch. Path. 46:179-187, 1948.

90. Rebuck, J. W., and Crowley, J. H., Method of Studying Leukocytic Functions *in vivo*, Ann. New York Acad. Sc. 59:757-805, 1955.

91. Spitznagel, J. K. and others, Cationic Proteins and Antibacterial Properties of Infected Tissues and Leukocytes, Amer. J. Path. 43:697-711, (Oct.) 1963.

92. Duff, G. E. and others, Uptake of Colloidal Thorium Dioxide by the Arterial Lesions of Cholesterol Atherosclerosis in the Rabbit, Am. J. Path. 34:941-951, 1958.

93. Rutstein, D. E. and others, Effects of Linolenic and Stearic Acids on Cholesterol-Induced Lipoid Deposition in Human Aortic Cells in Tissue Culture, Lancet 1:545, 1958.

94. Schayer, R. W., Significance of Induced Synthesis of Histamine in Physiology and Pathology, Chemotherapia 3:128-136, 1961.

95. Spain, D. M. and Aristizabal, N. J., Rabbit Local Tissue Response to Triglycerides, Cholesterol and its Ester, Arch. Path. 73:82-85, 1963.

96. Adams, C. W. and others, Phospholipids in Atherosclerosis: The Modification of the Cholesterol Granuloma by Phospholipid, J. Path. Bact. 86:431-436, 1963.

97. Harman, Denham, Atherosclerosis, Hypothesis Concerning the Initiating Steps in Pathogenesis, J. Gerontology 12: 199-202, 1957.

98. Dixon, K. C., Fatty Deposition: A Disorder of the Cell, Quart. J. Exp. Physiol. 43:139-158, 1958.

99. Day, A. J., Removal of Cholesterol from Endothelial Cells, Brit. J. Exp. Path. 41:112-118, 1960.

100. Zilversmit, D. B. and others, The Synthesis of Phospholipids in Human Atheromatous Lesions, Circulation 23:370-375, 1961.

101. _____, The Origin of Aortic Phospholipid in Rabbit Atheromatosis, Circulation 9:581-591, 1954.

102. Newman, H. A. and Zilversmit, D. B., The Origin of Various Lipids in Atheromatous Lesion of Rabbits, Circulation 20:967, 1959.