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Physical-Chemical Basis of Lipid Deposition in Atherosclerosis

The physical state of the lipids helps to explain lipid deposition and lesion reversal in atherosclerosis.

Donald M. Small and G. Graham Shipley

Atherosclerosis, the major cause of death in the United States, is characterized by focal fatty thickening in the inner aspects of large arterial vessels supplying blood to the heart, brain, and other vital organs. These lesions obstruct the lumen of the vessel and result in ischemia of the tissue supplied by the vessel. Prolonged or sudden ischemia may result in a clinical heart attack or stroke from which the patient may or may not recover.

For more than a century, scientists have associated the atherosclerotic lesions with the accumulation of lipids, specifically cholesterol and its esters, in the inner layers (intima) of large arteries. Many investigators have studied the metabolism and transport of cholesterol and have shown, for example, that increased cholesterol in the blood is related to an increased prevalence of coronary artery disease and heart attack (*1*). Much of the recent work in the field of atherosclerosis has been centered either on the characterization and metabolism of the specific serum lipoproteins which transport cholesterol and its esters (*2*) or on the histology, chemical composition, biochemistry, and metabolism of the cells and chemical components of arterial walls (*3*). Little attention has been paid to the physical state of cholesterol and its biologically important esters, and no effort has been made to relate this physical state to that of lipids in the normal or diseased human arterial wall (*4*).

The fact that large quantities of certain lipids (especially cholesterol, cholesterol esters, or phospholipids) accumulate in atherosclerotic lesions and, furthermore, that the rate of exchange of cholesterol between atherosclerotic

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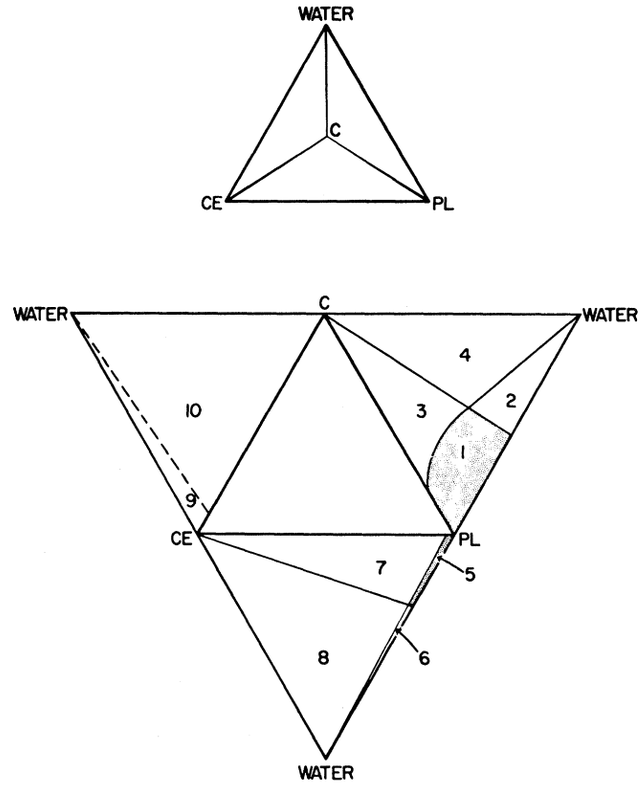

Fig. 1. The four-component system, phospholipid (PL), cholesterol ester (CE), cholesterol (C), and water, at 37°C and 1 atmosphere pressure. The amounts of the components present are expressed as percentages by weight. The tetrahedron (top) illustrates the classical representation of a four-component system whereby each apex represents 100 percent by weight of a single component, and any point within the tetrahedron represents a mixture of a specific composition. (Bottom) For the purpose of illustrating the three-component systems described in the text, the tetrahedron has been spread open from the water apex. On the right is the three-component system, PL-C-water. Zone 1, a single phase, the lamellar liquid-crystalline phase containing up to 33 percent by weight of C; 2, a two-phase zone consisting of hydrated lamellar liquid crystals of PL and C, and free water; 3, a two-phase zone containing liquid crystals of PL and C in equilibrium with crystals of C; 4, an invariant three-phase zone consisting of liquid crystals of PL saturated wth C and water, free water, and C crystals. At the bottom is the three-component system, PL-CE-water. Zone 5, a single phase of lamellar liquid crystal containing up to 2 to 3 percent CE; 6, a two-phase zone of lamellar liquid crystal and free water; 7, a two-phase zone, lamellar liquid crystal saturated with CE in equilibrium with CE; 8, an invariant three-phase zone consisting of lamellar liquid crystals saturated with CE, CE, and free water. At the left is the three-component system, CE-C-water. Zone 9, a two-phase zone consisting of an oily phase of CE containing up to 8 percent of C and free water; 10, an invariant three-phase zone consisting of an oily phase of CE saturated with C, C crystals, and free water.

plaques and plasma is slow (*5*) suggests that a major fraction of the lipids of atherosclerotic lesions exist in thermodynamically stable systems and that lipid-lipid interactions are the predominant force governing the physical state (solid, liquid crystal, liquid) of the lipid deposits (*6*). Thus we have studied the physical state of, and interrelationships between, the different lipid classes which accumulate in the lesions of atherosclerosis. The lipid-lipid interactions have been expressed in the form of condensed phase diagrams, and the compositional limits and structure of each phase have been determined by x-ray diffraction, calorimetry, and polarizing microscopy. By plotting the lipid composition of intimal lesions at different stages of severity on a phase diagram describing overall intimal lipid-lipid interactions we are able to predict the number and the physical state of the lipid phases that should be present in the lesions. To prove that these lipid phases actually exist in intimal lesions we have examined fresh human atherosclerotic lesions, of varying severity, by polarizing microscopy and x-ray diffraction and have confirmed the presence of the predicted lipid phases. Thus our studies indicate that the physical state of the lipids in normal and atherosclerotic intima is primarily determined by lipid-lipid interactions and that the lipid composition of the lesion determines what phases will be present in the lesion. Finally, a knowledge of lipid composition of intima at different stages of severity of atherosclerosis enables us to suggest some mechanisms for the growth and the regression of the lipid portion of the atherosclerotic plaque.

The lipid composition of the normal, young aortic intima in man consists mainly of phospholipids such as lecithin, sphingomyelin, and cephalins. Free cholesterol is also present but cholesterol esters are either absent or present in only trace amounts (*7, 8*). During the development of the early fatty streak lesion, cholesterol esters become increasingly abundant with the severity of the lesion (*7*). In the larger, more advanced fatty and fibrous plaques the overall concentration of lipid further increases, and cholesterol and its esters predominate (*7, 9–11*). Therefore, the three lipid classes with which we must concern ourselves are the phospholipids, free cholesterol, and cholesterol esters.

Lipid Phase Behavior

The physical state of these individual lipid classes in water is well known. Phospholipids, such as lecithin (*12, 13*) or sphingomyelin (*13–15*), swell in water to give a lamellar, liquid-crystalline phase consisting of planar arrays of lipid, two molecules thick, separated by layers of water. These liquid-crystalline phases undergo order-disorder transitions to produce more ordered lipid structures at low temperatures (*16*). Furthermore, mixtures of phospholipids obtained from biological origins, such as red blood cells or brain (*17*), hydrate in a similar fashion to give lamellar liquid-crystalline phases. On the other hand, free cholesterol is virtually insoluble in water and, when present in biological systems where it cannot be appropriately solubilized, it precipitates as cholesterol monohydrate—for instance, in cholesterol gallstones (*18, 19*) or in atherosclerotic lesions (*19*). Similarly, the cholesterol esters are completely insoluble in water (*4*).

How do these various lipids behave when mixed together in aqueous systems? The pertinent interrelationships can be shown by a complex four-component phase diagram in which the components, phospholipid, cholesterol, cholesterol ester, and water, are at a fixed temperature of 37°C and a fixed pressure of 1 atmosphere. Such a quaternary phase diagram is represented as a regular tetrahedron (see Fig. 1, top). For the purposes of defining the physical state of such lipids in relation to the environment of the intima, we need only concern ourselves

with the interactions of these lipids in an aqueous system containing an excess of free water, since the intima system does contain free water. Thus, the anhydrous three-component system, phospholipid-cholesterol–cholesterol ester, that is, the base of the tetrahedron, need not be discussed. The other three faces of the tetrahedron, shown as the unfolded sides of the tetrahedron in Fig. 1, are ternary systems and we have used phase equilibrium techniques, particularly light microscopy, differential scanning calorimetry, and x-ray diffraction, to characterize in detail the structure and interactions of these three-component systems. The main features of these phase diagrams are described below.

Phospholipid-cholesterol-water system. The equilibrium phase diagram of phospholipid (egg lecithin) and free cholesterol in water (*20*) shows that, except at low water concentrations (less than 12 percent by weight), mixtures of the three components form a single lamellar liquid-crystalline phase (zone 1, Fig. 1). When more than about 40 percent water is present (zone 2) two phases are present, the liquid crystals containing about 40 percent of water in excess water. The maximum amount of cholesterol that can be contained within the lamellar liquid-crystalline phase formed by lecithin is 1 mole of cholesterol per mole of lecithin, or about 33 percent by weight. Mixtures containing more than 1 mole of cholesterol per mole of lecithin separate another phase, cholesterol monohydrate crystals (zones 3 and 4). Lamellar phases of synthetic dipalmitoyl lecithin and sphingomyelin isolated from bovine brain show a similar limited swelling behavior in the presence of water, and both lipids can incorporate cholesterol up to equimolar ratios into this lipid-water phase (*15, 21*). The incorporation of cholesterol into these structures perturbs the molecular packing of the fatty acid moieties of the phospholipids and influences the temperature-dependent order-disorder transition shown by these lipids in the absence of cholesterol. Recently, we have confirmed that mixtures of pure phospholipids resembling those of normal aortic intima (45 percent egg lecithin, 45 percent sphingomyelin, 10 percent cephalin) behave in a fashion exactly analogous to egg lecithin, and can solubilize a maximum of one molecule of cholesterol per molecule of mixed phospholipid.

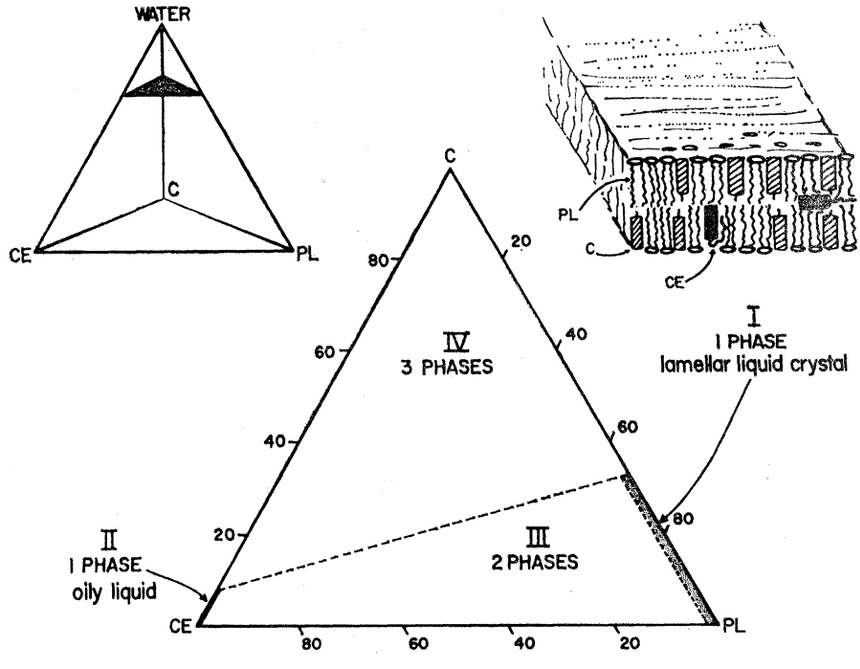

Fig. 2. The three-component system PL-CE-C at constant water content. The tetrahedron at the upper left shows the position of the section containing the four-component system with 70 percent of water by weight. This section is shown enlarged below, and is dealt with as a three-component system PL-CE-C (see text). Zone I, a lamellar liquid-crystalline phase containing varying amounts of C and CE (shown diagrammatically in the upper right); zone II, an oily liquid phase of CE containing up to 8 percent of C; zone III, a two-phase zone consisting of the lamellar liquid-crystalline phase and the oily liquid phase of CE; zone IV, an invariant zone of three phases, the lamellar liquid-crystalline phase saturated with C and CE, the oily CE phase saturated with C, and C crystals.

Phospholipid-cholesterol ester-water system. The interactions of lecithin with two biologically important cholesterol esters, cholesteryl linolenate and cholesteryl linoleate, in the presence of water have been studied at temperatures from 0° to 50°C by microscopic, calorimetric, and x-ray diffraction techniques (*22*). At 37°C, lecithin in an excess of water can incorporate very small amounts of cholesteryl linolenate, or cholesteryl linoleate, approximately 2 to 3 percent by weight, into its lamellar liquid-crystalline phase (Fig. 1, zone 5). Except at low water concentrations, when more than 2 to 3 percent cholesterol ester is present, the excess ester separates as an oily phase (Fig. 1, zones 7 and 8). In the presence of water, lecithin is not soluble in either the oil or liquid-crystalline phases formed by cholesterol esters.

Cholesterol ester-cholesterol-water system. The most abundant esters in serum lipoproteins (*2*) and arterial lesions (*7–11*) are cholesteryl linoleate and cholesteryl oleate. The binary systems of cholesterol–cholesteryl linoleate and cholesteryl oleate have been studied as a function of temperature (*4*). The addition of water to these systems as a third component affects neither the temperature nor the enthalpy of the phase transitions. At 37°C the solubility of cholesterol in the liquid oil phase of cholesteryl linoleate is 8 percent by weight (*23*). Thus the mixtures in zone 9 (Fig. 1) separate into two phases, an oily cholesterol ester phase containing variable amounts of free cholesterol, and water. Mixtures in zone 10 contain three separate phases, the cholesterol ester phase containing cholesterol, water, and crystals of cholesterol.

Lecithin-cholesterol ester-cholesterol-water system. Since the arterial intima is basically an aqueous system containing about 60 to 70 percent of water by weight, we will discuss the interactions of phospholipids, cholesterol esters, and free cholesterol in mixtures containing 70 percent of water by weight. The interrelationships of these lipids are represented on a triangular section of the tetrahedron taken parallel to the base where the concentration of water is 70 percent by weight (see Fig. 2, upper left). The Gibbs phase rule (*24*) states that at a specified temperature and pressure the variance (v) of a system is equal to the number of components (c) minus the number of

phases (p); that is, $v = c - p$. In the quaternary system at 37°C and 1 atmosphere of pressure, $v = 4 - p$. If the water concentration is fixed at 70 percent, the variance of the system is given by the equation $v = 3 - p$, where p represents *only* those phases containing one or more of the three components: phospholipid, cholesterol ester (cholesteryl linoleate), and free cholesterol. Thus the section taken where the concentration of water is 70 percent may be treated as a three-component system, phospholipid-cholesterol ester-cholesterol (*25*). From phase equilibrium studies we have found that four major zones exist in this system. Zone I (Fig. 2) consists of a lamellar liquid-crystalline phase of varying composition. The variance of the system is 2, and thus the concentration of two of the three components must be defined in order to fix the characteristics of the phase. This lamellar liquid-crystalline phase is formed by phospholipids but can incorporate up to one molecule of cholesterol per molecule of phospholipid plus a very small amount of cholesterol ester. The amount of incorporated cholesterol esters does not exceed more than 2 to 3 percent by weight of the total lipid. Zone II (Fig. 2) consists of an oily cholesterol ester phase containing up to 8 percent by weight of free cholesterol, but no phospholipid. In zone III (Fig. 2) two phases coexist: the oily cholesterol ester phase and the lamellar liquid-crystalline phase. The variance in zone III is 1. Finally, in zone IV, above the dashed line in Fig. 2, three phases of invariant composition are present. These phases are the oily cholesterol ester phase saturated with free cholesterol, the lamellar liquid-crystalline phase saturated with both cholesterol and cholesterol ester, and crystals of cholesterol monohydrate.

Lipid Phases of Arterial Intima

Are structures that are analogous to these lipid phases found in normal or diseased intimal tissue? First, membrane structures, such as plasma membranes, are more complex than the lamellar liquid-crystalline systems described above, but they probably possess a bimolecular leaflet of lipids, consisting primarily of phospholipids and free cholesterol, as a major structural component. In normal cell membranes the cholesterol to phospholipid molar ratio does not exceed 1, the maximum value exhibited by the simple lipid system. We predicted that the cellular membranes of the intima would not contain significant amounts of cholesterol esters but rather, if present in significant amounts, they would separate as an oily phase. We therefore expected that intimas having an overall lipid composition falling in zone I of Fig. 2 would behave as if the lipids were in a single bimolecular membrane phase. One would not expect to see the separation of either crystalline cholesterol or oily cholesterol ester phases.

On the other hand, in fatty streak lesions of the intima, we, and others (*26, 27*), have observed that some of the lipid is present as optically isotropic or birefringent oily droplets. Finally, light microscopy and x-ray diffraction studies of fresh aortic plaques (see Fig. 3) confirm the presence of both birefringent oily droplets and crystals of cholesterol monohydrate within these plaques (*19*). Therefore, the three

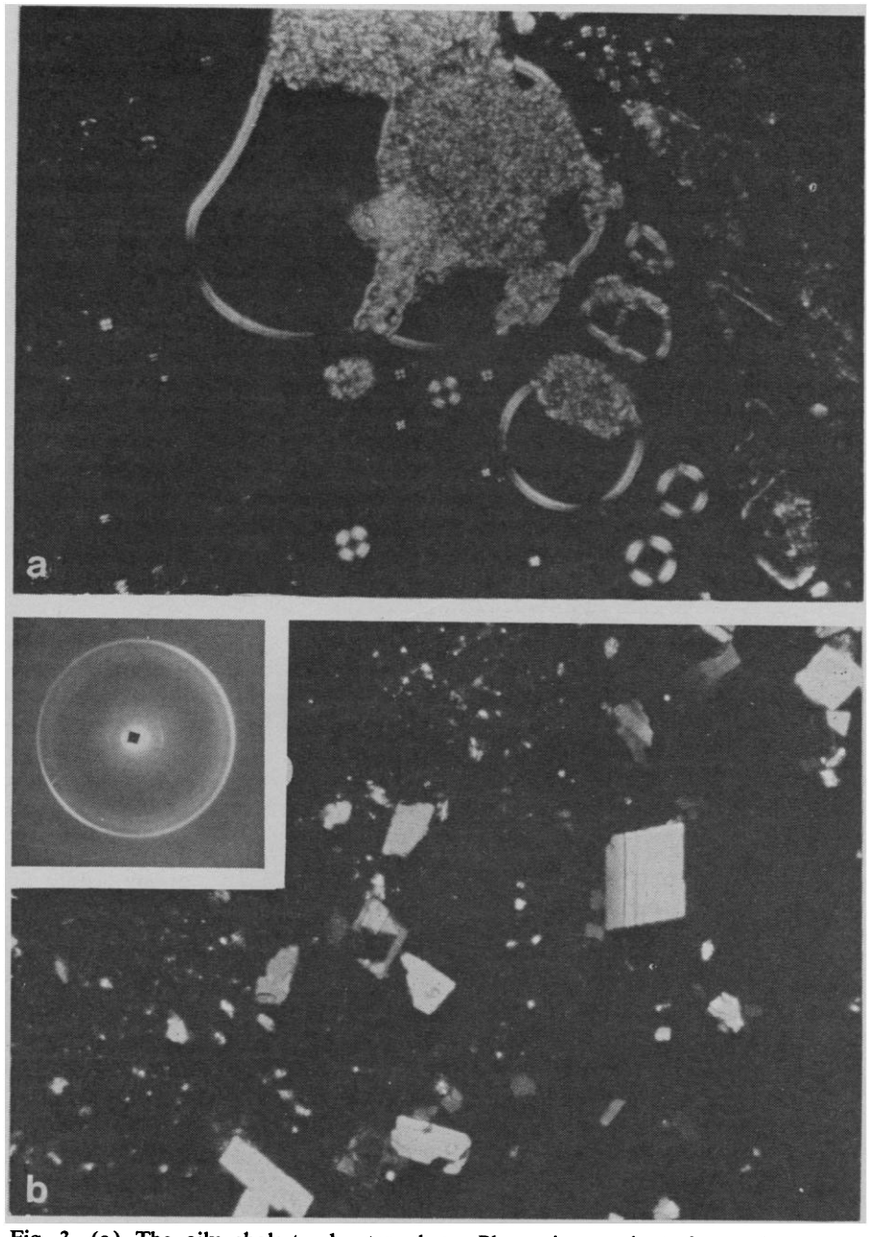

Fig. 3. (a) The oily cholesterol ester phase. Photomicrograph (×230) of a microdissected layer of a fresh advanced atherosclerotic plaque from the aorta of a 76-year-old woman showing the presence of numerous birefringent (optically positive) oily liquid crystal droplets at 23°C. These liquid crystal droplets melt to an isotropic oil between 37° and 40°C. (b) Photomicrograph (× 210) showing cholesterol monohydrate crystals isolated from the same plaque. The x-ray diffraction pattern (inset) from this plaque shows diffraction spacings consistent with the presence of cholesterol monohydrate crystals.

physical states (lamellar phase, oily phase, and crystalline cholesterol monohydrate) of lipids predicted from the quaternary phase diagram of a model system of the component lipids have been identified in aortic intimal tissue.

Phase Equilibria of Intimal Lipids

Can we predict the physical state of lipids within the arterial intima if we know, by experimentation, the lipid composition of the intima? To answer this question we have plotted, in Fig. 4, the compositions of normal aortic intima and related disease-affected regions on the phase diagram as presented in Fig. 2. Schematic representations of the histology of normal or diseased intima are shown on the right, and the lipid compositions in terms of total intimal phospholipid, cholesterol ester, and cholesterol (percent by weight) are plotted on the phase diagrams on the left. The intimal composition of prepubertal human aorta, calculated from the data of Smith *et al.* (*7*), is shown in Fig. 4a, point 1. Intimal lipids from human aorta, age 0 to 5 years, have almost identical compositions but the composition of such lipids from an older group shows a somewhat higher cholesterol level (*8*). Thus, at this early age the phospholipid bilayer is not yet saturated with cholesterol and there is little or no cholesterol ester present. Using polarizing light microscopy, we have been unable to find oil droplets, oily liquid-crystalline phases, or crystalline material in such intimas. Thus, all the lipid is in the lamellar or membrane phase as predicted by the phase diagram. In the normal intima taken from postpubertal males, age 15 to 20 years (*7, 28*), the composition (Fig. 4a, point 2) tends toward saturation of this phase with cholesterol and cholesterol ester. We have examined by polarizing microscopy fresh unfixed intimal tissue and found no recognizable liquid-crystalline phases, crystals, or oil droplets. Thus, it would appear that, although the membrane composition has changed, the lipids are still in a single phase, and presumably membrane bound. Since so little cholesterol ester is present, it is very difficult to detect by microscopy if there is a trace of a second phase formed by the ester. Points 3 and 4 in Fig. 4a were calculated from the work of Smith *et al.* (*7*) and represent lipid compositions of "normal" intima from groups of increasing age. As age increases the normal intima contains relatively more cholesterol ester. However, the fact that the intimal lipid composition falls in the two-phase region (zone III, Fig. 2), and almost on the boundary between the two- and the three-phase zone, shows that intimal lipids now exist in two phases: membranes nearly saturated with cholesterol, and an oily phase of cholesterol ester also saturated with cholesterol. The composition of the low-density lipoprotein lipids in serum (*7*) is shown for comparison in Fig. 4a (point 5).

Early fatty streak lesions (see Fig. 4b) were identified histologically and chemically by Smith *et al.* (*7*), who referred to these lesions as perifibrous lipid, since microscopically it is extracellular and appears to lie near fibrils of elastin. We observed by polarizing light microscopy that, although many of the lipid droplets are isotropic at 37°C, some are birefringent and melt to an isotropic liquid between 37.0° and 42.5°C. These droplets represent the oily cholesterol ester phase (zone II, Fig. 2) in the perifibrous microscopic fatty streak lesion. Like the lipids of the "normal" intima taken from persons over 20 years of age (Fig. 4a, points 3 and 4), as age increases, these lesions also become richer in choles-

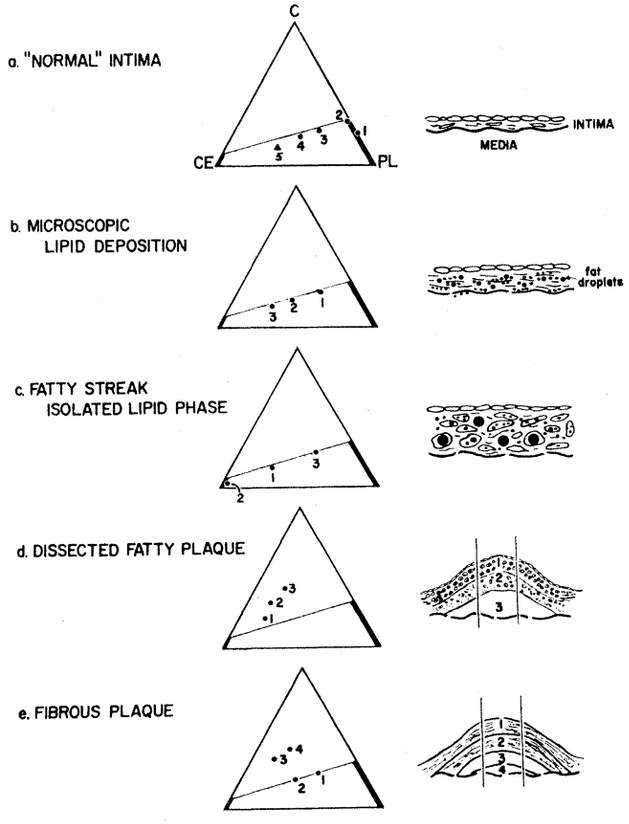

Fig. 4. The composition of normal and diseased human arterial intima plotted as percentages by weight on the three-component phase diagram, PL-CE-C. Schematic representation of the intima or intimal lesions are shown on the right. Since the lesions shown in (d) and (e) are comparatively large, they are less magnified than those shown in (a), (b), and (c). In (d) and (e), the points in the phase diagrams correspond to the regions shown in the diagrams on the right. (a) "Normal" intimal lipid composition as related to age: 1, prepubertal; 2, age 15 to 20 years; 3, age 20 to 30 years; 4, age 40 to 50 years; 5, the composition of low-density lipoproteins in serum, shown for comparison. [Data from Smith *et al.* (*7*)] (b) Microscopic lipid deposits. These primarily extracellular droplets were called perifibrous lipid by Smith *et al.* (*7*). The diagram on the right shows small fat droplets scattered among the fibrils of the intima. The intimal lipid compositions are plotted as a function of age: 1, under 30 years of age; 2, age 40 to 59 years; 3, over 70 years. (c) The fatty streak, according to Lang and Insull (*27*), is a macroscopic lesion which is visible to the naked eye and has both extracellular and intracellular fat globules. Point 1, lipid composition of the total fatty streak; 2, composition of the small fat globules floating up after centrifugation in water; 3, composition of the sedimenting residue. (d) Dissected fatty plaque: 1, the fat-filled nondegenerated cells occurring in the upper layers of these raised lesions; 2, the partly degenerated cells found in the next level; 3, the "amorphous" atheroma lipid. [Compositional data taken from Smith and Slater (*10*)] (e) Fibrous plaque: 1, surface layer of fibrous cap; 2, deeper layers of fibrous cap with abundant perifibrous lipid; 3, upper layer of "amorphous" atheroma lipid; 4, lower layer of "amorphous" atheroma lipid. [Compositional data taken from Smith and Slater (*10*)]

terol ester (Fig. 4b, points 1, 2, and 3). The two phases which are present are predicted to be a membrane phase saturated with both cholesterol and cholesterol ester, and a cholesterol ester oily phase saturated with cholesterol.

As the total quantity of lipid in the intima increases, larger lesions, called fatty streaks, are observed by the naked eye as yellow, flat, or slightly raised intimal lesions. Stewart (*26*) and Lang and Insull (*27*) observed both isotropic and birefringent lipid droplets in freshly dissected fatty streaks. The latter authors attempted to separate these droplets from the fatty streak by flotation centrifugation of homogenized intimal fatty streak. The lipid compositions of the whole fatty streak, the separated droplets, and the residue are shown in Fig. 4c. The droplets contain less than 2 percent phospholipid (Fig. 4c, point 2). We have noted that these droplets do not coalesce when they come in contact, suggesting that they are stabilized on their surface and can be considered as emulsion droplets. The amount of phospholipid necessary to form a monolayer at the surface of these droplets would account for the phospholipid composition of the droplets. Thus, the composition of these droplets demonstrates that Lang and Insull (*27*), using centrifugation, isolated the oily cholesterol ester phase in an emulsified state (*29*). The predominant lipid phase of the residue is the saturated membrane phase; however, the lipid composition of the residue (Fig. 4c, point 3) indicates that it still contains some of the oily phase. We therefore conclude that in the fatty streak lesion, lipids exist in at least two phases: (i) as a lamellar or membrane phase saturated with cholesterol and cholesterol ester [this phase must be saturated since its composition lies along the line which gives the limit of cholesterol saturation (dashed line, Fig. 2)]; and (ii) as either an oil or thermotropic liquid-crystalline phase formed primarily by the cholesterol ester. Although these lesions contain large amounts of lipid, we would not predict from the phase diagram that free cholesterol crystals would be present. In fact, we have not observed cholesterol crystals in this type of lesion.

Finally, the careful microdissection work of Smith and Slater (*10*) has led to the chemical definition of distinct histological regions in the advanced plaques of atherosclerosis. Although two different types of plaques have been described in terms of their gross histological features, the fatty plaque and the fibrous plaque, the majority of plaques probably contain aspects of both types. The fatty plaque (Fig. 4d) consists of lipid-filled cells in the upper layer (Fig. 4d, region 1), degenerating cells in the middle layer (Fig. 4d, region 2), and "amorphous" atheroma lipid in the lower layers (Fig. 4d, region 3). Lipids comprise a major portion of the dry weight of these lesions. For instance, the upper layer is about 30 percent lipid, the middle layer 40 percent, and the lower layer 65 percent. The composition of the upper layer (Fig. 4d, point 1 in the phase diagram) falls in the zone where three lipid phases might be expected. Thus, we would expect that, in addition to the membrane phase and the oily cholesterol ester phase, a third phase, crystalline cholesterol, would be present. However, it is possible that the excess cholesterol in these cells is maintained in a supersaturated state in either of the other two phases. Since no data exist on the physical state of lipids in this region of the plaque, either the coexistence of three phases, or the presence of two phases, one of them containing cholesterol in a supersaturated state, are reasonable possibilities. In region 2, the composition (Fig. 4d, point 2) moves toward the cholesterol apex, suggesting that more free cholesterol should be present as a third phase. The "amorphous" atheroma lipid (*10*) of region 3 has still more free cholesterol (Fig. 4d, point 3). We have observed that the base of these lesions does indeed contain cholesterol crystals, as well as an oily lipid phase. We would predict that lipids present in this region of the plaque would partition into at least three coexisting phases: cholesterol crystals, an oily cholesterol ester phase saturated with cholesterol, and a membrane phase saturated with cholesterol and cholesterol ester (*30*).

The fibrous plaque can be divided into at least four regions, as shown in Fig. 4e. These four regions include the surface layer of the plaque (Fig. 4e, region 1) which has a low lipid content and contains large amounts of fibrous tissue, a deeper layer (region 2) containing extracellular lipid, and basal regions 3 and 4 which contain large amounts of lipid of the "amorphous" atheroma type. The composition of each of these regions, as given by Smith and Slater (*10*), is plotted on the phase diagram in Fig. 4e. The upper fibrous layers, which contain some perifibrous lipid, as determined by histological techniques, lie along the line separating the two phases (Fig. 4e, points 1 and 2). In fact, their relative lipid composition is similar to that of the perifibrous lipid shown in Fig. 4b. Thus we predict that the lipids coexist in two phases; the lamellar membrane phase saturated with cholesterol, and a second oily cholesterol ester phase. However, the "amorphous" parts of the plaque (Fig. 4e, points 3 and 4) lie well above the line separating zones III and IV (Fig. 2), suggesting that they should have free cholesterol precipitated as crystals. When we examined the "amorphous" parts of advanced plaques by polarizing microscopy we consistently found, in addition to the oily cholesterol ester phase, that cholesterol crystals were present (see Fig. 3). Perhaps the concept of "amorphous" lipid (*9*, *10*) may be misleading in the physical sense, in that at least some of the lipid is present in a crystalline form. The total lipid compositions of advanced plaques reported by many other investigators (*31*) are also located in the three-phase zone and lie well above the line separating the two- and three-phase zones.

Process of Lipid Deposition

We conclude that the physical state of the lipids in the plaque is determined primarily by lipid-lipid interactions. Thus, the number of lipid phases and their physical state can be predicted by the lipid composition. The fact that intimal lipid composition changes as lesions progress from a microscopic to a gross size allows us to predict, in physical terms, the sequence of lipid deposition in the arterial wall, as shown in Fig. 5. The first changes in the intima that occur with age are presumably an increase in the ratio of cholesterol phospholipid and to increase in the ratio of sphingomyelin to lecithin. Thus, intimal membranes in the first few years of life probably contain less than a full complement of free cholesterol and little or no cholesterol ester (Fig. 5, see point 1 and the corresponding schematic representations). For some obscure reason, cholesterol begins to increase in the membrane fraction where it appears to stimulate the compensatory synthesis of sphingomyelin or the transfer of sphingomyelin into

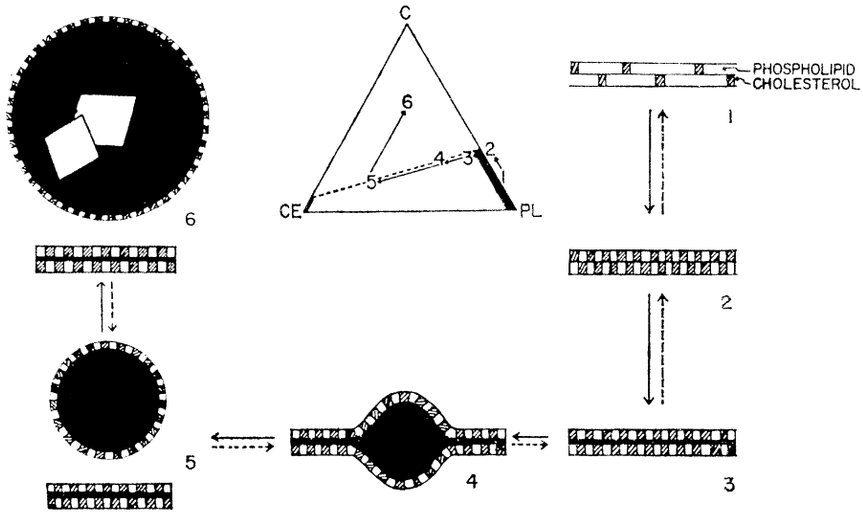

Fig. 5. Schematic representation of the progression of lipid deposition in the arterial intima. Points 1 to 6 in the phase diagram refer to corresponding schematic representations. (1) In childhood, lipids exist in a single lamellar phase associated primarily with the plasma and other cellular membranes of the intimal cells. Cholesterol concentration is relatively high compared to some other plasma membranes but has not yet saturated the membrane phase and little or no cholesterol ester is present. (2) With age, perhaps after puberty, cholesterol saturates the membrane system and, (3), cholesterol ester saturates the membrane phase. (4 and 5) When excess ester is present it separates as a separate phase, oily cholesteryl ester–rich droplets covered with a surface layer of phospholipid and cholesterol. (6) The proportion of free cholesterol increases and ultimately leads to the precipitation of cholesterol crystals as a third phase in the more advanced plaques. This solid phase has been identified principally in the lower layers of advanced fatty and fibrous plaques. Although reversibility of the lipid lesions is theoretically possible, the rate of reversal may depend in large part on which lipid phases are present and on the composition of these phases.

membranes. As the amount of sphingomyelin increases, so also does the amount of membrane lipid (relative to DNA content), as demonstrated in intimal tissue of subhuman primates (*32*). However, as cholesterol reaches maximum saturation in the membrane, it appears to stimulate cholesterol ester formation, perhaps as a mechanism to divert the insoluble and potentially crystalline cholesterol into a less toxic liquid form. Thus, there are two compensatory processes that may help to maintain the cholesterol in a noncrystalline state: one is the increased membrane production, which may be caused primarily by the increased sphingomyelin; and the other is the increased accumulation of cholesterol ester, perhaps as a result of the increased esterification of free cholesterol. When the membrane becomes saturated with both cholesterol (Fig. 5, point 2) and cholesterol ester (Fig. 5, point 3), the ester separates as a second lipid phase, either as oil droplets or as one of the oily thermotropic liquid-crystalline phases formed by cholesterol esters. Small amounts of free cholesterol are solubilized in this phase. As the lesion progresses, the total amount of the oily phase increases relative to that of the membrane phase; that is, the lipid composition changes from that at point 3, through the compositions at points 4 and 5 in Fig. 5. Factors other than cholesterol esterification might be responsible for the increase in the amount of the oily ester phase, especially the deposition of part or all of the lipids of low-density lipoproteins. It should be noted that the lipid composition of this class of lipoproteins (Fig. 4a, point 5) is very similar to that of fatty streaks (Fig. 4c, point 1). Finally, for unknown biochemical reasons, the amount of free cholesterol increases above its solubility in either the membrane phase or the oily cholesterol ester phase and thus produces an unstable condition. In the basal regions of advanced fatty and fibrous plaques, the excess cholesterol precipitates out as cholesterol monohydrate. Although the specific metabolic derangements leading to these physical changes are not understood, we think that future metabolic studies might be more productive if they were viewed within the framework of knowledge of the physical state of the lipids of the normal and diseased arterial wall.

Reversibility of Atherosclerotic Lesions

Knowledge of the physical state of lipids in the lesions of the arterial wall allows us to make predictions concerning the possible reversibility of atherosclerotic lesions. First, the early changes in intimal lipid composition (Fig. 5, points 1, 2, and 3) may represent an early part of the aging process. It is not known whether these changes are reversible in a biochemical sense. The early fatty lesions, such as those shown in Fig. 4a, points 3 and 4, and Fig. 4b, points 1, 2, and 3, are characterized by the accumulation of cholesterol ester and some free cholesterol in an oily liquid or liquid-crystalline phase (Fig. 2, zone II). Similar oily phases rich in cholesterol ester are known to accumulate in such organs as the adrenal (*33*) and the corpus luteum of the ovary (*34*). The cholesterol ester in these phases can be rapidly metabolized. Although the intima of the aorta may not have all the appropriate enzyme systems and transport apparatus necessary, it is conceivable that the lipids of the cholesterol ester–rich phase could be metabolically removed. If the removal rate exceeded the accumulation of new molecules, then reversal of this type of lesion would be possible. In the case of the advanced plaque, however, lipids exist in at least three phases and the third phase, cholesterol crystals, presents a distinct problem in removal. If the crystal is surrounded by the oily phase, then this phase must become unsaturated with respect to cholesterol to promote dissolution of the trapped crystals. If the cholesterol crystals are not in contact with the oily phase, their dissolution will depend upon unknown and complex factors related to the removal of molecules from the crystal surface in an undefined milieu.

Thus, we predict that the reversibility of advanced lesions may be limited to removal of the oily phase of plaque and that dissolution of crystalline deposits may be minimal in the presence of a cholesterol-saturated oily phase. Only if the oily phase can be made unsaturated with respect to cholesterol can there be a net movement of cholesterol from the crystal to the oily phase. Future studies on the composition of the plaque and especially of the oily phase during attempts at reversal of experimental lesions, for instance in primate models, may answer the questions regarding the mechanism of reversal and the degree to which advanced plaques may be reversed.

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30. We fully realize that this may be an oversimplification. First, cholesterol esters such as cholesteryl palmitate, cholesteryl stearate, and cholesteryl oleate, which are known to exist in plaques (*9*), have melting points well above 37°C (*4*) and although these esters may form low-melting eutectics with more highly unsaturated esters [such as the eutectic noted with cholesteryl linoleate–cholesteryl oleate systems (*4*)] it is possible that some of these esters might exist as crystalline solids in the plaque. Esters in a crystalline form have not, however, been identified in fresh plaques. Second, sphingomyelin can form an ordered phase above 37°C, especially if its proportion to the other membrane phospholipids becomes large. Thus, since sphingomyelin becomes the major phospholipid in advanced lesions it is possible that an ordered phase of sphingomyelin could be formed and be present in the plaque.
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Analogy as a Source of Knowledge

Konrad Z. Lorenz

Concept of Analogy

In the course of evolution it constantly happens that, independently of each other, two different forms of life take similar, parallel paths in adapting themselves to the same external circumstances. Practically all animals which move fast in a homogeneous medium have found means of giving their bodies a streamlined shape, thereby reducing friction to a minimum. The "invention" of concentrating light on a tissue sensitive to it by means of a diaphanous lens has been made independently at least four times by different phyla of animals; and in two of these, in the cephalopods and in the vertebrates, this kind of "eye" has evolved into the true, image-projecting camera through which we ourselves are able to see the world.

Thanks to old discoveries by Charles Darwin and very recent ones by biochemists, we have a fairly sound knowledge of the processes which, in the course of evolution, achieve these marvelous structures. The student of evolution has good reason to assume that the abundance of different bodily structures which, by their wonderful expediency, make life possible for such amazingly different creatures under such amazingly different conditions, all owe their existence to these processes which we are wont to subsume under

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