The pathogenesis of atherosclerosis¹

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Atherosclerosis is characterized histologically by lipid accumulation and a variable connective-tissue reaction. This constitution suggests that important considerations in atherogenesis are (a) the cause of sclerosis, (b) the cause of lipid accumulation and failure of its removal, and (c) the origin of the lipids.

Sclerogenic Mechanisms

Duguid (1946, 1952) extended Rokitansky's earlier observations by suggesting that encrustation of fine platelet deposits and films of fibrin on the endothelium provokes an inflammatory response that results in organization by scarring. This view has been modified by supposing that local fibrinolytic activity in the arterial wall may control the equilibrium between encrustation and solution of fibrin (Astrup, 1959). Although Duguid's mechanism does not in itself explain how lipid enters the arterial wall, a subsequent suggestion is that vasoactive compounds from encrusted platelets increase endothelial permeability and thus permit lipid to enter (Mustard, 1967, 1970; Constantinides and Robinson, 1969).

HAEMODYNAMIC STRESS

Stresses imposed by the pulsatile nature of the blood flow would be expected to promote reparative changes in the tunica intima, a process akin to Virchow's 'reparative endarteritis' (Aschoff, 1924). This sort of stress would be expected to pull the tunica intima forwards in the direction of flow on the underlying tunica media (Duguid, 1926; Adams, 1964; French, 1966) and to strain the elasto-muscular organization of the tunica media (Wolinsky and Glagov, 1964).

A number of haemodynamic hypotheses have been proposed to explain the focal distribution of atherosclerosis. The locally disturbed flow pattern at the orifices of branch vessels is presumed to be responsible for the intensification of the disease at these sites. Indeed, thymidine autoradiography (Wright, 1971, 1972) and the dye-exclusion test

(Björkerud and Bondjers, 1972) show that cells proliferate more rapidly and endothelial permeability is increased around these junctions.

Oscillatory wave reflection from large bifurcations, eg, the aorto-iliac junction, has been proposed as the cause of the severe atherosclerosis that is seen in the lower human abdominal aorta: such disease is associated with a progressive expansion (mismatching) of aortic calibre compared with that of the common iliac arteries (Newman, Gosling, and Bowden, 1971).

High shear promotes endothelial damage and increased permeability to dyes (Fry, 1968, 1969). Likewise, hypertension is an important epidemiological factor in ischaemic heart disease (Dawber, 1962) and accelerates the development of experimental atherosclerosis (Heptinstall, Bankley, and Ponter, 1958).

CHOLESTEROL-INDUCED SCLEROSIS

Lipids implanted under the skin induce granuloma formation (Spain and Aristizabal, 1962). The most potent lipids for inducing sclerosis are cholesterol, its more saturated esters, and certain free fatty acids (Abdulla, Adams, and Morgan, 1967). Certain degradation products of cholesterol, eg, cholestenol and cholestanol, and implants of mixed atheroma lipids also provoke severe sclerosis. The sclerogenic potential of cholesterol and its esters is related to their hydrophobic character, and can be ranked: monenoic esters > free cholesterol and saturated esters > dienoic esters > tetraenoic esters. By contrast, phospholipids lower the surface tension of cholesterol and other hydrophobic lipids (Zilver-smit *et al.*, 1954); they disperse and accelerate the resorption of hydrophobic sterol compounds (Adams, 1967; Adams and Morgan, 1967); they also prevent the sclerogenic action of such sterols (Adams, Bayliss, Ibrahim, and Webster, 1963).

Lipid Accumulation

NATURE OF ATHEROMA LIPIDS

Many investigators have shown that cholesterol, particularly its ester, is the predominant lipid to accumulate in atherosclerotic lesions (Böttcher,

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1964; Smith, 1965). The proportion of cholesterol as ester increases during the development of atherosclerosis (Smith, Evans, and Downham, 1967; Lofland, St. Clair, Clarkson, Bullock, and Lehner, 1968) but declines again when the lesion 'regresses' (McMillan, Horlick, and Duff, 1955; Adams, Morgan, and Bayliss, 1972). Phospholipids increase in the early stages of the lesion but triglycerides are never more than minor components (Smith, 1965; Adams, Bayliss, Abdulla, Mahler, and Root, 1969; Abdulla, Adams, and Bayliss, 1969). The relative absence of triglycerides is probably due to the protection afforded by the arterial wall lipase (Zemplényi, 1962, 1968); this enzyme retains its activity even in old age (Adams *et al.*, 1969).

Perhaps the most important cause of cholesterol accumulation in the arterial wall is that the sterol cannot be significantly metabolized therein, apart from minor degradation or esterification. The liver can excrete cholesterol and degrade it to water-soluble cholic acids, but other tissues—apart from some endocrine glands—lack this capacity to deal with this damaging sterol.

CHOLESTEROL ESTERIFICATION

Although it is not certain which enzymatic routes are most concerned in the esterification of cholesterol in the arterial wall, such local esterification is mainly directed towards the formation of cholesterol oleate (Bowyer, Howard, Gresham, Bates, and Palmer, 1968; St. Clair, Lofland, and Clarkson, 1968). Thus, the arterial wall is in this connexion its own worst enemy, for this ester is one of the most sclerogenic of the lipids we tested (see above). Nevertheless, only about one-fifth of the esterified cholesterol in atheroma is derived from local synthesis, the rest is more unsaturated and its fatty acid pattern is similar to that of the plasma esterified cholesterol fraction (Smith, 1965; Smith, Slater, and Chu, 1968).

There is some disagreement whether cholesterol is mobilized from the atheromatous artery in free or in esterified form. Tissue culture studies suggest that cells only absorb and secrete cholesterol in its free form (Rothblat and Kritchevsky, 1967). However, macrophages seem to be capable of ingesting both free and esterified cholesterol without preliminary hydrolysis of the latter (Adams, Abdulla, and Bayliss, 1971). A reasonable conclusion would be that cholesterol is transported across endothelial cells predominantly or exclusively in free form, but that its resorption by phagocytic cells is effected by pinocytosis—both free and esterified forms would be equally susceptible to this process.

ACIDIC MUCOSUBSTANCES

Gerő, Gengely, Dévényi, Jakab, Székeley, and

Virág (1960) originally suggested that acidic mucosubstances (glycosaminoglycans) in the arterial wall may entrap lipoprotein by forming an insoluble complex with it; this idea was later confirmed by Bihari-Varga and Végh (1967). A number of pathologists have also reported on the association between lipid deposits and acidic mucosubstances in atherosclerotic lesions; these reports have been discussed by Walton and Williamson (1968). Hitherto, however, the relationship between the lipid and the mucosubstances in histological sections has not been established, the suggestion being that the mucosubstances seem to be arranged around the lipid rather than under or in it. This can nevertheless easily be explained: the lipid of the lipoprotein forms a hydrophobic or greasy layer over the mucosubstance and prevents dyes penetrating and staining it. Preliminary lipid extraction allows the relevant dyes to penetrate and stain the underlying mucosubstances (Adams and Bayliss, 1973).

ISCHAEMIA OF THE MEDIA

The arterial wall is not an inert piece of plumbing for it has an active metabolism of its own. With advancing age the tunica intima of certain human arteries becomes diffusely thickened. This process seems to be a proliferative repair process and is distinct from atherosclerosis. This diffuse intimal thickening impairs the diffusion of nutrients of small molecular size from the lumen. Normally the inner two-thirds of large human vessels is nourished by direct permeation from the lumen, while the outer third is nourished by the vasa vasorum in the tunica adventitia (Kirk and Laursen, 1955). With the advent of intimal thickening the mid-zone of the tunica media becomes ischaemic, and later this process extends inwards to affect the inner media. The ischaemic damage to the human aortic media has been detected by histochemical and microbiological means (Adams, Bayliss, and Ibrahim, 1962; Adams, 1967; Zemplényi, 1968; Adams and Bayliss, 1969; Hoff, 1970). The effect of this ischaemia on the aorta would be to impede the outflow of cholesterol from the endothelium towards the adventitia, either by causing a structural block or by depressing the endogenous synthesis of protein and other lipotropic agents. Reduced outflow of cholesterol through the tunica media would promote accumulation in the tunica intima, as is seen in atherosclerosis.

Origin of the Lipids

ENTRY OF LIPID AND PROTEIN

The phospholipids of atherosclerotic lesions are at least in part synthesized locally (Zilversmit and

McCandless, 1959; Stein, Stein, and Shapiro, 1963; Zilversmit, 1970). By contrast, although sterols are synthesized by the arterial wall, this source seems to make a negligible contribution to the cholesterol deposited in the lesions and most of it comes from the blood (Zilversmit, 1968; Lofland and Clarkson, 1970). Such cholesterol synthesis as does occur in the arterial wall is more likely to be related to the formation of cell membranes.

Virchow (see Aschoff, 1924) first suggested that plasma constituents are imbibed by the structurally altered arterial intima. Page (1954) extended this filtration hypothesis by supposing that β - (low-density) lipoprotein is unstable and sheds its load of cholesterol after entering the arterial intima. The presence of such lipoprotein has been demonstrated in the normal and atherosclerotic human artery by electrophoretic, immunofluorescent, and radioisotope techniques (Gerö, Gengely, Jakab, Székeley, and Virág, 1961; Kao and Wissler, 1965; Woolf and Pinkington, 1965; Walton and Williamson, 1968; Scott and Hurley, 1970; Smith and Slater, 1970, 1972). Likewise, the fatty acid pattern of the cholesterol esters in atherosclerotic lesions is consistent with a major origin from plasma rather than by endogenous synthesis (Smith, 1965; Smith *et al*, 1968).

In spite of this identification of β -lipoprotein in the arterial intima, experimental evidence shows that cholesterol enters the normal vessel mainly in free form and not as ester (Newman and Zilversmit, 1962, 1966; Hashimoto and Dayton, 1966). In view of Rothblat and Kritchevsky's (1967) work mentioned above, the preferential entry of free cholesterol into the arterial wall may simply reflect transport of cholesterol in this form across the endothelium. Nevertheless, the preponderant entry of free over esterified cholesterol counts strongly against the direct entry of significant amounts of β -lipoprotein into the normal arterial intima, for this lipoprotein carries a two- to three-fold excess of the ester. Studies with ^{125}I -labelled albumin and globulin in normal and mildly atheromatous rabbits show that these proteins normally enter mainly from the outer surface of the aorta (Adams *et al*, 1969): their molecular size is much smaller than that of β -lipoprotein, so they would presumably enter more easily than the lipoproteins. However, with the development of severe atheroma (plaques > 0.1 mm thick) the labelled albumin gradient reverses and most enters from the inside (Adams, Morgan, and Bayliss, 1970). This observation suggests that damage to the endothelium in established atheroma permits major entry of lipoprotein from the lumen. By implication atheroma or atherosclerosis may be a self-perpetuating process once it has exceeded a certain critical phase.

Although much protein—and by inference lipoprotein—seems to enter from the intimal surface in the atheromatous artery, cholesterol enters the atheromatous vessel by two routes. (1) Labelled cholesterol rapidly enters the outer part of the rabbit aorta, but much of this radioactivity rapidly declines, particularly in less severely diseased vessels (Adams, 1971). This relatively fast entry may represent the rapid circulation of lipoprotein from the adventitial vessels through the outer part of the wall. It does not seem to be comparable with the rapid uptake of particulate cholesterol by the reticuloendothelial system, recently described by Nilsson and Zilversmit (1972). In any case this seemingly fast entry into the outer wall is almost certainly unimportant in the process of atherogenesis. (2) Labelled cholesterol enters somewhat more slowly into the inner part of the rabbit aorta, but radioactivity does not subsequently decline. A slower phase of cholesterol entry is also seen in the outer part of the rabbit aorta—particularly in severely atheromatous vessels (Adams and Bayliss, 1973).

These results can be summarized as follows:

- 1 Most protein—and by inference lipoprotein—enters the normal rabbit aortic wall from the outer surface.
- 2 Most protein enters severely atheromatous aortas from the inner surface, probably as a result of endothelial damage.
- 3 Cholesterol enters the atheromatous aorta from both the inner and outer surfaces. Much of the entry from the outer surface is rapid and transient. Entry from the inner surface is slower but cumulative.

CHOLESTEROL TURNOVER

We have reviewed elsewhere the evidence concerning the reversibility of atherosclerosis (Adams, 1967, 1973; Adams *et al*, 1973). In man severe malnutrition appears to cause at least partial resorption of the lipids in atherosclerotic lesions. No certain evidence of regression of atheroma has been obtained from studies on experimental animals except that minor lesions, caused by very brief periods of cholesterol feeding, regress when cholesterol is stopped (Bortz, 1968). The only certain change seen in the experimental animal after stopping a cholesterol-enriched diet is a progressive increase in fibrous tissue, so that the atheromatous lesion is converted with the passage of time to an atherosclerotic one (Anitschkow, 1933; see Adams *et al*, 1973). Histochemical methods and lipid analyses show no significant change in the cholesterol content of rabbit aortic atheromatous lesions—apart from a proportional decrease in the ester fraction—for up to a year after stopping a cholesterol-enriched diet (Adams *et al*, 1973). Likewise the specific activity of labelled

The pathogenesis of atherosclerosis

cholesterol in the aortic intima remains relatively unaltered for all of this period, once the aortic level has reached that in plasma.

This metabolic inertia of cholesterol in atheroma may be caused by the breakdown of lipoprotein that has infiltrated the aortic intima. The following is a simplified analysis of possible compartments or pools in the atheromatous artery (Adams, 1973):

Lipoprotein in Plasma (1) $\rightleftharpoons$ Lipoprotein in Arterial

Pools 1 and 2 might be expected to equilibrate rapidly, so that a fall in pool 1 would fairly quickly lead to a fall in pool 2. However, once the lipoprotein has been denatured, cholesterol would no longer be bound to its apoprotein vehicle and would be deposited in the tissues as extra-cellular masses and crystals. Cholesterol in this pool 3 would presumably be physically inaccessible for exchange processes and, being extracellular, would also be inaccessible to cellular metabolism and transport processes. Such deposited cholesterol would thus have little if any turnover and would be difficult to absorb. Whether or not severe malnutrition would open up pool 3 so that it becomes more accessible for resorption remains a matter for conjecture. Experience in World War II suggests that gross malnutrition would have this effect in man, but the less severe malnutrition resulting from planned therapeutic semi-starvation may be less effective.

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Addendum

Since this paper was written considerable regression has been described in atheromatous coronary arteries of rhesus monkeys, first fed on atherogenic diet then returned to a low-lipid and corn-oil-enriched diet (Armstrong and Megan, 1972).

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