The origins of atherosclerosis

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Summary

The presence of atherosclerotic lesions in young adults suggests that early stages of atherogenesis occur during childhood. The relationship of intimal lesions in childhood to fully developed atherosclerosis is briefly discussed. Factors likely to promote lipid accumulation within the arterial wall and proliferation of connective tissue elements are reviewed with particular emphasis on endothelial cell injury and the possible consequences of this for intimal smooth muscle cell proliferation.

LESIONS which have all the hallmarks of atherosclerosis can be found in major blood vessels as early as the third decade of life in a wide range of post-mortem samples. Even discounting circumstances such as familial Type II hyperlipidaemia, which appears to be causally related to precocious atherosclerosis, it is likely that the factors – endogenous or environmental – which are concerned with atherogenesis, operate during childhood. It is with these factors, the responses of the different elements of the arterial wall and the ‘early’ lesions which are produced, that this brief review is primarily concerned.

Despite a vast literature we are still largely ignorant of much of the essential nature of atherogenesis. However, it seems generally accepted that two processes which are fundamental to plaque formation are: the proliferation of modified smooth muscle cells within the arterial intima; and the accumulation of intra- and extra-cellular lipid – most of which seems to be derived from the plasma. Before we examine some facets of these processes and the factors which affect them, we should consider some of the morphological aspects of what are commonly called ‘early atherosclerotic lesions’, their epidemiological relationship to the future development of clinically significant arterial disease, and the anatomical background against which they occur.

The fatty streak

Macroscopic deposits of lipid can be found in the intima of large elastic arteries such as the aorta from early infancy onwards and have been recorded in

the aorta of fetuses (Sinzinger *et al.*, 1975). In the aorta and coronary arteries they appear initially as minute yellowish dots minimally elevated above the surface of the surrounding intima. These dots then coalesce to form the streak lesion so commonly seen at post-mortem. It seems quite likely that blood flow patterns have some influence on the topography of these lesions since fatty streaks occurring just above the aortic valve ring tend to lie roughly at right angles to the long axis of the aorta instead of parallel to this axis as seen more distally.

The light microscopic and ultrastructural appearances of the fatty streak have been excellently described (Geer and Haust, 1972) but controversy still clouds the relationship, if any, that may exist between the fatty streak and the raised lesions which constitute atherosclerosis (Robertson, 1967). Part of the difficulty in resolving the question may arise from the distinct probability that a number of morphologically distinct lesions may shelter under the general umbrella of ‘fatty streak’.

McGill (1974) has described at least three varieties of fatty streak. The first of these, which is the dominant arterial lesion in childhood and adolescence, is found in all population groups. Socio-economic circumstances and the liability of adults in the population to develop atherosclerosis appear to be without influence on its occurrence. The lipid in this ‘juvenile’ fatty streak is predominantly intracellular and there is little or no formation of new connective tissue. In adolescence the lesions increase rapidly in number and are more extensive in females and among Negroes. Both these points strongly suggest that the ‘juvenile’ fatty streak is a poor predictor of the development of raised lesions.

A second variety of fatty streak occurs in young adults, especially in those who belong to population groups in which there is a high background level of atherosclerosis. This lesion contains much of its lipid in the form of extracellular accumulations and in areas where intact cells are scanty. In other parts of these lesions numerous cells are present and some of these undergo necrosis. An increase in the extracellular components of connective tissue is also apparent. It has been suggested (McGill, 1974) that

this type of fatty streak may progress and constitute a precursor of the fibro-lipid atherosclerotic plaque. The relevance of the fatty streak to fully developed atherosclerotic plaques needs to be reassessed in the light of these morphological differences, although the amount of labour involved in such a study would be immense.

The gelatinous lesion

Increasing attention has recently been directed to the possible role of small blister-like elevations of the intima as precursors of atherosclerotic plaques (Haust, 1971a, b). Since these lesions are translucent they are easily overlooked on cursory examination and no data exist relating to their frequency and very little to their distribution (Smith, 1975). On light microscopy their appearances are rather banal consisting essentially of oedematous separation of intimal collagen and elastin. On biochemical analysis, the intimal content of plasma-derived constituents is considerably increased, there being about twice as much albumin and about four times as much fibrinogen and lipoprotein as in the 'normal' intima. The ultimate fate of these lesions and their place in the atherosclerotic canon is not yet clear and may well remain so until more data on their frequency, topography and age distribution have been collected.

Growth and remodelling in the artery wall

The lesions described above occur against the background of rapid and striking changes in the arterial intima which start to occur as early as the 34th week of gestation (Neufeld, Wagenvoort and Edwards, 1962). In the fetus before this time and in many infants in the first few weeks of life, the arterial intima shows a relatively simple structure, only a few sheets of connective tissue separating the endothelial lining from the internal lamina elastica.

Within a short space of time, the internal lamina elastica appears to split and form new laminae and smooth muscle cells appear between the separated elements of the elastic laminae with the formation of a fairly easily discernible new layer, the musculo-elastic layer. Diffuse intimal thickening, more marked, for example, in the abdominal than the thoracic aorta, is a universal concomitant of ageing and may indicate no more than a normal adaptive process to changing dynamic demands rather than constitute a precursor for atherosclerosis. However, the great increase in intimal thickness may well influence or modify the process of atherogenesis and is certainly accompanied by a considerable increase in the concentration of plasma low-density lipoprotein, and other plasma constituents, within the artery wall (Smith and Smith, 1976).

The coronary arteries of infants and young

children frequently show focal intimal thickening as well as the diffuse variety of which mention has already been made (Minkowski, 1947; Moon, 1957, Wolkoff, 1923). The precise significance of these changes is not clearly understood, but Vlodaver, Kahn and Neufeld (1969) showed that the degree of intimal thickening in the coronary arteries of children from three different ethnic groups correlated with the reported prevalence of clinical coronary artery disease in adults of these three groups.

The lipid content of the intima

Plaque lipids consist of cholesterol and other minor sterols in free and esterified form, phospholipids and a small amount of triglyceride. It is important to bear in mind that these focal accumulations of lipid occur in an arterial wall which contains lipid, even in lesion-free areas; the amount and nature of this lipid changes with increasing age.

The total amount of intimal lipid in infants and young children is small but with each decade there is an increment of about 0.2 mg/100 mg dry tissue in the content of free cholesterol, total phospholipid and triglyceride. Morphologically, such lipid as can be demonstrated by Sudan staining appears as a fine extracellular stippling, the droplets being aligned in relation to connective tissue fibres, especially to fragmented elastic laminae (Smith, Evans and Downham, 1967).

The pattern of accumulation of cholesterol ester in the lesion-free intima differs from the slow but steady increase in free cholesterol which takes place during the first two decades. Esterified cholesterol remains almost constant at a level of about 0.4 mg/100 mg of defatted, dried tissue over most of the first two decades, but then begins to rise steeply at a rate almost five times as fast as free cholesterol (Smith, 1974). In addition to this sudden 'take off' in cholesterol ester levels there are characteristic changes in the fatty acid patterns from infancy onwards. In the youngest age groups studied by Smith, the cholesterol esters were characterized by a high proportion of C.16 acids and a low proportion of C.18:2 (linoleic) acid. There are also higher proportions of both long- and short-chain minor components and of C.18:0 (stearic) acid. This type of fatty acid pattern is said to be characteristic also of the serum cholesterol esters in infants and very young children (Zee, 1968). As the child grows changes occur in the intimal fatty acid pattern with a rapid increase in the amount of the 18:2 as compared with the 18:1 fraction (Smith, 1974).

Qualitatively, therefore, Smith found that arterial cholesterol ester seemed to be in equilibrium with plasma cholesterol ester although there was no net accumulation of ester within the wall. Even more interesting in this connection was the fact that, in

Smith's studies of the arterial intima during the second decade of life, the amount of extractable lipoprotein could account for only about one-third of the cholesterol ester.

Lipid accumulation with lesions

In intimal lesions between 65–80% of the lipid fraction is cholesterol and the ratio of esterified to free sterol is high in all types of lesion (Böttcher, 1963). The fatty acid composition of fatty streak esters differs markedly from that found in fibro-lipid plaques and in plasma (Geer and Guidry, 1964; Smith, 1965). In the streak the cholesterol esters contain a high proportion of oleic acid and relatively small amount of linoleate. These data have been interpreted as suggesting esterification of cholesterol *in situ*, probably within the smooth muscle cells of the arterial intima; the presence of the ester of eicosatrienoic acid (normally not found in plasma) within the streak lends substance to this view.

The origin of plaque cholesterol

Turnover studies of labelled cholesterol suggest that the bulk of the cholesterol in the human aorta is derived from the plasma (Field *et al.*, 1960). Since this sterol is transported in the plasma in intimate association with one of the ion apo-protein carriers, there is obviously some importance as to whether the cholesterol enters the artery wall as lipoprotein. There is good evidence both from immuno-fluorescent studies (Watts, 1961; Kao and Wissler, 1965; Woolf and Pilkington, 1965; Walton and Williamson, 1968) and from microimmunoassay techniques (Smith and Slater, 1972) that the lipoproteins are present both in macroscopically normal intima and in atherosclerotic lesions. In lesion-free areas of the aortic intima there is a significant correlation between the intimal concentration of low-density lipoprotein and plasma cholesterol (Smith and Slater, 1972).

The mode of transport of the macromolecular lipoprotein across the endothelial barrier has excited considerable interest. Under normal circumstances it seems likely that lipoproteins are transported across intact endothelial cells by means of micro-pinocytosis (Stein and Stein, 1973). Whether this mechanism is responsible for increased amount of low-density lipoprotein accumulating within the intima during atherogenesis is not known, and certainly other mechanisms involving damage to or loss of endothelium could also be involved. The majority of investigators in this area have concentrated on the question of influx of cholesterol across the arterial endothelium: efflux of sterol from wall to plasma can also take place (Dayton and Hashimoto, 1966) and the intriguing suggestion has been put forward that local inhibition of diffusional efflux

from wall to blood may play a major controlling role in differential localization of lipid deposits especially in relation to arterial branches. The proposed mechanism through which efflux could be regulated consists essentially of differences in wall shear stresses, and is apparently independent of boundary layer thickness (Caro, Fitzgerald and Schroter, 1969, 1971; Weinbaum and Caro, 1976).

Endothelial permeability and endothelial injury

The fact that plasma lipids and other plasma constituents increase focally within the arterial wall during atherogenesis suggests that this process must be accompanied either by focal increases in endothelial permeability or by a decline in the efficiency of mechanisms which 'clear' the plasma derived molecules from the arterial intima. There is ample evidence of focal increases in the permeability of the endothelial barrier (Somer and Schwartz, 1971; Bell *et al.*, 1973) and in the pig, at least, such areas show increased endothelial cell turnover (Caplan and Schwartz, 1973). In experimental animals, focal increased permeability and lipid deposition appear to go hand in hand. The logical correlate of this situation must then be to consider the spectrum of endothelial cell changes which may account for the changes in permeability.

Most of our knowledge in this area comes, perforce, from experimental animals. Endothelial injury, using the word in a broad sense, may be related to blood-flow patterns and Fry (1973) showed that shearing stresses in excess of 400 dyn/cm² cause deformation of endothelial cells and, ultimately, erosion from the sub-endothelial tissues with consequent platelet adhesion and aggregation.

FIG. 1. Scanning electron micrograph of rabbit aorta $\times 420$. Rabbit fed on cholesterol-rich diet for 3 months. Several focal endothelial defects are present and macrophages and red cells adhere to the luminal surface.

Similarly, sensitivity of the endothelial cell to its chemical milieu has been shown in a number of experimental systems involving different noxae such as homocystine (Harker, Slichter and Scott, 1974), high cholesterol diets (Fig. 1) (Ross and Glomset, 1976); cigarette smoke (Frost, 1973; Asmussen and Kjeldsen, 1975). A single dose of norepinephrine given to an anaesthetized rabbit produces striking changes in the aortic endothelium as viewed with the scanning electron microscope (Fig. 1) and even hypoxia during perfusion may produce defects in the plasma membrane of the cells. Since denudation of endothelium not only creates new pathways for the ingress of plasma lipids but is also inevitably associated with some degree of platelet adhesion, a study of factors which may damage the endothelial barrier during early life, when the seeds of subsequent atherosclerosis are, no doubt, being sown, merits considerable attention.

Proliferation of modified smooth muscle cells

Without proliferation of modified smooth muscle cells within the arterial intima, it is unlikely that the atherosclerotic plaque as we know it could develop at all. Not only do the cells constitute the most significant element of the cell population of the plaque but recent studies have shown that they are also capable of synthesizing the extracellular connective tissue matrix which is such an important part of the plaque substance (Ross, 1973; McCullough and Balian, 1975; Wight and Ross, 1975; Narayan *et al.*, 1976).

Any injury to the arterial wall which involves a significant loss of endothelial cells is followed inevitably by adherence of platelets to sub-endothelial structures. An integral part of the natural history of these lesions is a marked degree of intimal smooth muscle proliferation. Woolf *et al.* (1968) found, for example, that gentle abrasion of the luminal surface of the pig aorta, which is comparable in thickness to that of a young child, resulted in focal increases of the intima of up to twelve times normal in less than three weeks. This intimal thickening is due to a striking degree of smooth muscle proliferation, the new cells being arranged in regular layers roughly parallel to the luminal surface of the vessel wall.

Is this striking increase in smooth muscle and, in due time, of its products a non-specific 'repair' process, or are there aspects which make this process unique to the arterial intima? The feature associated with the arterial injury which makes it different from injury in other situations is the involvement of platelets. Unless platelet number and function are normal the fibro-muscular proliferation which follows experimental injury is inhibited (Harker *et al.*, 1976; Moore *et al.*, 1976). In this connection, Ross, Glomset and Harker (1977) quote a study in

which it was reported that spontaneous atherosclerosis is markedly reduced in pigs with von Willebrand's disease as compared with 'normal' control animals.

Support for the concept that the platelet plays a central role in this proliferation phase of atherosclerosis has come from the work of Ross and his colleagues (Ross and Glomset, 1976; Rutherford and Ross, 1976) who showed that platelets release a low-molecular-weight basic protein which has the capacity to trigger the proliferation of arterial smooth muscle in culture.

Is smooth muscle proliferation a response to injury?

Until now there appears to have been tacit agreement that the proliferative phase of atherosclerosis represents a reaction to injury using the word in a broad sense. A fascinating new view has emerged from the studies of Benditt and Benditt (1973). The proposition that these workers have put forward is that smooth muscle cell proliferation with fibrous plaques is monoclonal in type, and that the process is thus more closely related to what occurs in smooth muscle tumour formation than in the repair phase of injury.

This approach stems originally from the discoveries of Lyon (1961) relating to random inactivation of an X chromosome in each normal mammalian female diploid cell and the fact that any female is, in a sense, a phenotypic mosaic. Under most circumstances this is of no practical significance since both X chromosomes code for similar enzymes. However, glucose 6-phosphate dehydrogenase (G 6-PD) is a special case since it can occur in two iso-enzymic forms. About 33% of Negro females in the U.S.A. are heterozygous for the enzyme. Individual cells from these females show either the A or B form of the enzyme but not both. In patches of macroscopically normal aortic intima from such women, both iso-enzymes are present. In the majority of fibrous plaques, however, only one iso-enzyme can be identified in any individual plaque (Benditt, 1977; Pearson *et al.*, 1975, 1977). In a recent publication Benditt (1977) suggested that some of the environmental noxae mentioned, e.g. cigarette smoking, may act as mutagens and influence smooth muscle proliferation in this way. Pearson *et al.* (1977) found that about 33% of fatty streaks from the arterial aortae of Negro females heterozygous for G 6-PD were of the single phenotype variety and may, by implication, constitute precursors of fibrous plaques.

Conclusion

In the foregoing an attempt has been made to draw attention to some of the mechanisms which may play a part in the genesis of atherosclerotic plaques. These processes must certainly operate

during childhood and adolescence since reliable post-mortem data indicate that atherosclerotic lesions are present in the large arteries in young people, especially those belonging to economically privileged communities. If the prevalence of atherosclerosis is to be diminished, then clearly we must direct our attention at an early stage of life to those factors, particularly exogenous ones, which may trigger the key functional and structural changes in the arterial wall which result in significant atherosclerosis.

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