Atherosclerosis: Introduction to Atherosclerosis

Updodate

Presented by Dr Barry Lewis, Department of Chemical Pathology, Royal Postgraduate Medical School; Professor Neville Woolf, Department of Histopathology, Middlesex Hospital Medical School; Professor Colin Adams, Department of Pathology, Guy's Hospital Medical School.

University of London Audio-Visual Centre, 1975.

Made for British Postgraduate Medical Federation.

Black and white

Duration: 00:38:38:15

00:00:00:00

<**Opening titles over shots of people walking in city street, traffic, exhaust fumes, cigarette smoking, sausage and egg frying in a pan, measurement of skin fat, a blood pressure gauge, cholesterol molecules, a crowd with a chunk of people missing from it**>

<**Dr Barry Lewis to camera**>

Atherosclerosis can manifest in various ways, the gravest of these are myocardial infarction, angina pectoris and, of course, sudden death itself. In this series we shall be dealing with its pathogenesis and also with present views on prevention of its complications. Ischaemic heart disease is usually, though I must emphasise not always, based upon coronary atherosclerosis. The frequency of ischaemic heart disease seems to be rising quite steeply in this country and in most Western countries.

<**Lewis refers to diagram on board on table next to him showing mortality statistics of ischaemic heart disease in the UK, then to camera**>

This graph shows the mortality statistics in the United Kingdom over the past 4 decades and you will see on this logarithmic scale an almost 8-fold increase in the frequency of death from ischaemic heart disease in men with an almost comparable increase in women. This scale of increase would be even more dramatic if the scale were not logarithmic.

Now, to some extent this may reflect diagnostic fashions: there is a greater tendency today to diagnose ischaemic heart disease than there was 4 decades ago. Nevertheless, the disease is increasing in some age groups in the present century, even in the present decade and also it is affecting younger and younger individuals. For that reason, I think, there is a general rise in the incidence of ischaemic heart disease. By death certificates it seems that about a quarter of all deaths are from ischaemic heart disease and in the United States more than half of men die of ischaemic heart disease today, even this is possibly an underestimate.

Now, the rise of frequency of the disease over recent decades establishes, I think, that environmental factors are important in the aetiology. Of course, this does not rule out an important role for genetic factors too and these will be reviewed by Dr Slack and Dr Lloyd in a later section. However, the pathogenesis of ischaemic heart disease obviously involves multiple factors; one of these, for example, involves hypertension, which Professor Dollery and his colleagues will be discussing in a later section. Another is diet, and this has been revealed over the past 20 or 30 years by the detailed work of Ancel Keys.

<**Lewis over graphs showing results of Ancel Keys study into diet and cholesterol levels in relation to ischaemic heart disease, to camera in between**>

This figure shows one of Keys' sets of data, in a study of 18 communities in variety parts of the world. He related the mortality statistics from ischaemic heart disease,

here, to the dietary intake of saturated fat, shown horizontally. And you will see how those communities with a greater intake of saturated fat also have a higher mortality rate, as exemplified by Eastern Finland here, with the highest fat and the highest mortality rate, and by these communities here, including Japan, in which fat intake and mortalities from heart disease are both very low.

Now, to some extent, this dietary effect seems to be mediated through hyperlipidaemia and this constitutes a dietary fat hypothesis which we shall be discussing in later sections. Here again is the work of Ancel Keys now relating the serum cholesterol concentration, the average serum cholesterol concentration in these various communities, to the mortality from ischaemic heart disease. And once again you can see this strong positive correlation in those communities having a high mean cholesterol having a greater mortality rate.

Now, the role of lipids is discussed in detail in later sections too. Today, however, we shall be discussing atherosclerosis itself, its pathology and its pathogenesis. With us in the studio is Professor Neville Woolf who will start by discussing the histopathology of the atherosclerotic lesion.

<**Professor Neville Woolf to camera**>

Since our understanding of the process of atherosclerosis is still so far from complete, we have to define the disease in morphological terms, however unsatisfactory this may appear.

<**Woolf over diagram, then photograph showing an atherosclerotic artery, then to camera**>

And within this frame of reference we can say, therefore, that atherosclerosis is the widely prevalent disease of large elastic and muscular arteries which is characterised by focal elevations of the inner lining of these arteries. And these focal elevations consist in part of basal accumulations of lipid and tissue debris which are separated

from the vessel lumen by a connective tissue cap containing large numbers of smooth muscle cells, collagen fibres and elastic fibres.

Let's now turn to the real thing from this diagrammatic representation. And here we can see part of the aorta of a middle-aged man. We see quite clearly the focal elevations, some are yellow because of a massive basal pool of lipid, some are greyer or pearly because the connective tissue cap is much thicker. And in others, you can see one in the lower left hand portion of the screen, ulceration has taken place with its inevitable consequence, thrombosis.

<**Woolf to camera, then over diagram showing the progress of atherosclerosis over lifetime**>

From the clinician's point of view, it's important to be aware that atherosclerosis is an iceberg disease. It's prevalent from the very early part of our lives, the ubiquitous fatty streak first appearing within the first 2 decades of life. By the time we are in our 20s, we begin to develop raised lesions, more archetypal of atherosclerosis though on a somewhat limited scale. And by the time we are in our 40s not only may we, in Western communities, have large numbers of these raised lesions but we now begin to have complications supervening on the established atherosclerotic plaque. The most important of these complications is undoubtedly thrombosis and it is at this point that the lesion surfaces above what some have called the clinical horizon and expresses itself as a series of either lethal or crippling events – myocardial infarction or sudden death, stroke, gangrene or aneurism formation.

00:07:44:00

<**Woolf to camera, then over slides showing microscopic depictions of atherosclerotic lesions, occasionally to camera between slides**>

Now, let's examine these lesions in their microscopic form. Here we have what is conventionally regarded as the earliest lesion of atherosclerosis, though this is a somewhat controversial point. This is a fatty streak, here. We see intimal thickening,

this is the internal elastic laminar here, and this localised area of thickening is characterised by the accumulation of very numerous cells, most of which are either smooth muscle cells or modified smooth muscle cells. And holes in the interstitial tissue, which, in this conventionally processed specimen, represent where lipid substances have been dissolved out. Whether this lesion really relates statistically to our epidemiological data regarding obtrusive arterial disease, or whether, indeed, it relates to the amount of fully developed atherosclerosis, which we see in later life, is still controversial.

However, there can be no doubt about the relationship of this lesion to the clinical situation. This is the raised lesion of fibrolipid plaque, the archetypal lesion of atherosclerosis and the one which epidemiologically is related to the differing prevalence and influence of arterial disease throughout the world. As you can see, there is now a massive thickening of the arterial intima which is thicker, in fact, than the rest of the arterial wall. At the base of the thickened intima, this section has been stained to demonstrate lipids, we see this dark staining, a massive pool of lipid substances separated from the lumen of the vessel by a fairly thin connective tissue cap.

The importance of these lesions is even greater in vessels of comparatively small calibre, such as the coronary arteries, which supply such a vital tissue as the myocardium and here, as we can see, an atherosclerotic plaque in one of the coronary arteries. The lumen of this vessel has been reduced to about a fifth of normal and the intima vastly thickened by this accumulation of lipid substances. Note also the secondary phenomenon associated with the intimal thickening and that is the marked thinning of the underlying muscular coat of the artery, an almost inevitable finding in relation to atherosclerotic plaques.

Sometimes we see something rather different, particularly in the coronary arteries, an appearance that makes us think that perhaps the growth of the plaque has been episodic. And in this rather archaeological specimen, resembling the Seven Cities of Troy, one upon the other, we see a connective tissue cap just under the lumen, a

basal pool of lipid, another connective tissue cap, another pool of lipid and so on and so on until eventually we come to the markedly thinned media.

This photograph here shows us the graphic representation of the coup de grace, not only for the lesion, but for the patient. Here we have our basal pool of atheromatous debris, rich in these cigar-shaped cholesterol crystals. And here, and there, the remains of the connective tissue cap, this has ruptured, sprung apart and the gap has been filled up with this mass of dark-staining thrombotic material. And when carefully examined, more than 90% of recently occluded coronary artery segments show this appearance.

00:12:25:00

<**Woolf to camera, then over table showing factors leading to a coronary clinical event, then back to camera**>

The last thing that we should be aware of is to avoid an over simplistic approach in stating that the extent of atherosclerosis is the only factor which relates to clinical arterial disease. It is one, and probably an important one, relating to the clinical event, to the myocardial infarct, to the stroke, to gangrene, but we must be aware that there are other aspects of this problem which must be examined. A susceptibility of affected tissue, the rate and the mode of the growth of individual plaques because a single necrotic plaque may produce just as devastating a clinical effect as a large number, the question of platelet functions in relation to occlusive neural thrombosis and the question of small vessel disease within the tissues supplied by the atherosclerotic vessel. All of these may form part of the ultimate clinical event.

Now, Professor Colin Adams is going to tell us something about the components of the atherosclerotic plaque and, in particular, the lipids which form such an important part.

<**Professor Colin Adams to camera, then over slides showing arterial lipid accumulation**>

The plaques are composed of two main things: the lipid and the connective lipid proliferation or the sclerosis or the scarring. We'll be talking in a moment or two about the scarring, let's start on the lipids.

As you can see here there's a massive accumulation of lipids in this vessel, a really vast amount in comparison with some of the more modest lesions, modest amounts, that one sees in the earlier lesions. Most of this is cholesterol which is present if one looks in polarised light, in these little rhomboidal and sometimes acicular crystals that we can see scattered around here. Modern work suggests that most of this is in fact free cholesterol, whereas the esterified cholesterol which is a major component of the plaque, using one of Dr Roy Weller's illustrations, is present as these liquid crystals, rather charming multi-coloured little footballs here, there, and in other parts. So this is the two forms in which cholesterol is present.

The chemistry, and here I'm using one of Neville Woolf's small chromatograms from tissue sections, it shows a very marked accumulation of free cholesterol and some ester cholesterol in contrast to the relatively little amounts in the normal vessel – you see there's much more here in atheroma than in the control vessel, and note, in particular, the absence of triglyceride and only relatively small amounts of phospholipids.

<**Adams, shown seated, refers to graph on board on table to his left, showing levels of lipids in blood over lifetime**>

This is a biochemical expression of the same thing. From the age of 20 up to the age of 90 we have a remarkable increase of cholesterol in the aortic wall. During this time phospholipids go up a little whereas triglycerides show a minimal or trivial increase. This, as we'll see later, with Barry Lewis we'll be discussing this point, is because there are enzymes in the arterial wall that can break down the triglycerides and phospholipases that break down the phospholipids, but there's nothing, absolutely nothing that the arterial wall can do to get rid of cholesterol in the chemical sense, it

cannot break it down, it hasn't got enzymes like the liver that can break cholesterol down into bile salts and similar compounds.

<**Adams to camera, then over slide showing how cholesterol causes atheroma scarring**>

Connective tissue proliferation is the other major feature of the atherosclerotic lesion. One cause of this proliferation of connective tissues and scarring is probably the irritant effect of cholesterol in the tissues.

This is an experiment where cholesterol was injected subcutaneously and it shows the amount of scarring, a vast amount of scarring, around the cholesterol implant. And I consider this to be one important reason for the scarring of atheroma. The other important reason is encrustation of films of fibrin on the endothelium and fine films of platelet and here I pass you back to Neville Woolf who's going to discuss this encrustation.

00:17:40:00

<**Woolf to camera, then over slides showing atherosclerotic plaques derived from thrombosis**>

I'd like now to consider the proposition that a significant part of the growth of an atherosclerotic plaque may derive from thrombosis on its surface with subsequent incorporation within the vessel wall. This is not a new hypothesis, it was first put forwards in 1856, but like the sleeping beauty it lay dormant for nearly 100 years until Professor Duguid, in 1946, gave it the kiss of life.

There are two ways in which we can examine this proposition. The first is to look at human lesions and to see within them whether we can identify the residua of thrombi. Everyone can do this in conventionally prepared material such as we see here, a section through an atherosclerotic block; stained with a connective tissue stain and moulded to the luminal surface of this plaque is a massive accumulation of fibrin and

platelets and, more interesting, separated from it and within the tissue cap, are these band-like aggregates of similarly staining material.

Occasionally, if one looks at areas where there's just a little bit of roughening of the aortic or coronary artery surface, one will see this. And again, one can see a thin layer of darkly staining materials which is mainly fibrin, separated from the lumen by a thin cap of collagen and smooth muscle cells. And, once again, deeper within this thickened intima, similar band-like aggregates of this material.

<**Woolf to camera, then over slides showing fibrin in atherosclerotic plaques**>

Now, conventional histological stains for plasma proteins are extremely capricious and we want something a little more accurate and a little more definite, and we find this in the use of antisera specially prepared against human plasma proteins and against native cells.

And in the next slide we see an atherosclerotic plaque which has been stained or treated with a fluorescent anti-fibrin serum and within the connective tissue cap and, indeed, on its surface as well, one can see these brightly fluorescent bands of fibrin lying roughly parallel to the luminal surface. And one can see this in 40 to 50% of all atherosclerotic plaques.

It's fair to say that there may be explanations other than the thrombotic one to account for the presence of fibrin within these lesions but there can be no other one to account for the presence of aggregated platelets. And in this section we have used an anti-human platelet serum, once again linked with a fluorescent dye, and you can see, separated from the lumen, these band-like aggregates of platelet antigen.

<**Woolf to camera, then over slides showing an artificially produced thrombus on an arterial wall**>

Let's now look at the other side of the medal, the experimental one, in which we produce a thrombus on a normal arterial wall in one or other animal species. My own

work has involved the use of the pig and in the next slide we see a massive fresh thrombus, thicker, as you can see very easily, than the underlying artery wall which has been produced by light abrasion of the intimal surface. This animal was allowed to survive for 3 days after the removal of the endothelium. If we're relatively merciful and allow the animal to survive for a month, you see that that massive thrombus has gone and we have now produced a quite respectable imitation of an atherosclerotic plaque with marked focal endothelial and intimal proliferation, causing a marked degree of intimal thickening, and a basal pool which consists in part of the residua of the thrombus and in part of lipid, the amount of lipid depending on the plasma lipid levels of the animal.

00:22:32:00

<**Lewis to camera**>

Lipids, then, form an important part of the atheromatous plaque. A number of research workers have addressed themselves to the very important problem of the origin of this plaque lipid. These include Colin Adams and Neville Woolf and their colleagues. It's clear that some phospholipid and a little cholesterol is synthesised locally within the arterial wall and Colin Adams has shown how labelled cholesterol injected into plasma rapidly appears in the arterial intima. Silversmith, however, has calculated that most of the plaque cholesterol comes from the plasma and is not that which is locally synthesised. Neville Woolf has shown, by immunohistochemistry that lipoproteins are present in the intima. It is these lipoproteins which transport the lipids in plasma.

<**Lewis, shown seated, refers to diagram on board to his right showing concentrations of lipoproteins in plasma related to atherogenesis**>

Dr Onitiri and I have been comparing lipoprotein concentrations in the plasma with those in the arterial wall. The samples we obtained simultaneously from patients undergoing arterial surgery. We measured the lipoproteins in the serum by using an antibody raised against plasma lipoprotein levels.

In this figure you'll see the concentrations of the two lipoproteins in plasma which have been related to atherogenesis. The low density, or beta lipoprotein, the very low density, or pre-beta lipoprotein. These dots indicate samples of arterial intima which have been analysed for lipoproteins and you will see that the concentration of lipoproteins is directly related to the concentration of plasma lipoprotein in the same individual. This is true both for the beta lipoprotein which carries cholesterol and for the pre-beta lipoprotein which carries triglyceride through plasma.

<**Lewis to camera, then over diagram showing how lipoproteins enter the intima**>

In view of this direct proportionality, it follows that the concentration of lipoproteins in plasma is a determining factor for the concentrations of lipoproteins in the atheromatous plaque. Now, we think that low density lipoprotein probably enters the intima intact but there has been some controversy over the importance of very low density lipoprotein or pre-beta lipoprotein. The controversy arises because VLDL (very low density lipoprotein) is a large molecule and not all workers believe that particles of this size could cross the endothelial barrier, at least when this barrier is intact.

However, Silversmith has recently produced the hypothesis that the importance of pre-beta lipoprotein is that it is metabolised by the enzyme which Colin Adams has referred to, lipoprotein lipase, which is situated in the endothelial surface and this enzyme removes much of the triglyceride from the molecule producing a progressively smaller molecule which is relatively enriched in cholesterol. And it is these cholesterol-enriched products which actually are small enough to cross the endothelial barrier. This would explain the relative absence of triglyceride from the atheromatous plaque.

Colin Adams has studied the ingress of lipids from plasma into the arterial wall experimentally. He will now tell us about this.

<**Adams to camera, then over slide of autoradiograph showing endothelium after injection of tritium cholesterol**>

It's possible to trace the fate of tritium cholesterol, radioactive cholesterol and iodine labelled proteins not only by micro-chemical methods using scintillation counting but also under the microscope by autoradiography.

Now, with these autoradiographs one can show that very early after injection of tritium cholesterol, of radioactive cholesterol, there's a localisation, little autoradiograph spots in the endothelium. And it would seem that endothelium is, in a sense, a rate-limiting process in that it takes time for the granules, or the cholesterol, to pass through this particular zone.

<**Adams, shown seated, refers to chart to his left showing a diagram of elastic lamina acting as a barrier to cholesterol, then to camera**>

Now, I would like to come next to thinking what other barriers that there are to the entry of cholesterol into the arterial wall. I've been talking here about the endothelium as a barrier. There's also, we must consider, the elastic lamina, these structures here, which tend to hold up lipoprotein entry because they only have these little small holes to let the lipoprotein through to get out on the other side of the vessel. Our producer here has very elegantly referred to this as a blot oil filter and I think this is an extremely good simile because once these little fenestrations are blocked by lipoprotein, it would tend to lead to the accumulation of lipid and lipoprotein in the inner part of the arterial wall at this point.

Now, coming to the whole matter of endothelial injury. One can look upon atherogenic mechanisms, such as we know about them, of course there are probably many that we know nothing of, one can look upon them, these atherogenic mechanisms, as various types of injury.

<**Adams refers to diagram showing atherogenic mechanisms as injury, then histochemical diagram using ATPase, then to camera**>

First of all there's the purely mechanical injury – a lot of work has been done on this by workers such as Donald Fry, showing that the stress of blood flow exerts a shear on the arterial intima, leading to connective tissue proliferation. The lipid that enters the arterial wall itself is fibrogenic, as we discussed earlier. Neville Woolf has shown that the encrusted thrombus and fibrin promotes a sub-acute inflammation that leads on to scarring, what the pathologists call organisation. There is also another injury to the arterial wall, that is that the mid-zone, here, becomes ischaemic when the tunica intima, the inner lining, thickens. You see the mid-part of the arterial wall is nourished by permeation from the lumen, here. The outer part is nourished by these rather large vasa, as shown in this diagram. But as your vessel, as your intima, thickens the mid-zone becomes hypoxic and we can show this in a diagram of a histochemical picture using ATPase. And this very mid-zone that I was showing suffers from a lack of diffusion of oxygen, even by the age of 29, and this man was only 29, shows a loss of ATPase from the smooth muscle, and this metabolic damage must surely limit the egress of lipoprotein out of the arterial wall.

This is clearly another injury that leads to accumulation in the inner part of the vessel. Now, Neville Woolf has other injuries to talk about, specific injuries to the endothelium; damage to the endothelium, of course, will tend to let things in.

00:30:08:00

<**Woolf to camera**>

I suspect that our thinking on this subject has been hampered by the models that we've tended to use. Quite clearly, for instance, scraping off the endothelium from the inside of an animal's aorta is not like anything that we imagine happens in real life. And we must, instead, look for injuries to the vessel wall which lie within normal daily experience, or normal daily biological experience. For instance, cigarette smoking is known to increase the mortality from occlusive arterial disease, and, it is strongly suggested, increases the amount of atherosclerosis. How may it do this? We know, for instance, that the carbon monoxide levels in the blood are higher in

cigarette smokers and that this produces changes in the endothelial cell. Similarly, cigarette smoking is followed by the production of catecholamines and, consequently, by a rise in free fatty acid levels in the blood. This again will produce blebs in the endothelial cells and may very well produce changes in the inter-endothelial junctions. I think, possibly, that we haven't been asking the right questions and that we're entering into a new phase where we must look very carefully at the role of thousands of times repeated small injuries of the kind that I have mentioned as probably playing a major role in atherogenesis, and in particular in the widely different degrees of atherogenesis that we find in different communities and in different parts of the world.

<**Lewis to camera**>

Lastly today, I would like to examine the possibility that atherosclerosis is reversible or, at least, that its thrombotic complications are reversible or can be arrested. Underlying this assumption are all the attempts to decrease the prevalence of ischaemic heart disease by treating raised lipids, hypertension and diabetes, and by persuading people to abandon cigarette smoking, for example.

Now we've known for years that ischaemic heart disease mortality abruptly dropped in Western countries at the start of the Second World War. We've also results of the trials of a fat-modified diet which lowers the serum cholesterol level. These trials all suggest that the frequency of ischaemic heart disease may be reduced over a period of 5 or 10 years by about 30-40%. Now unfortunately neither source of information shows specifically that atherosclerosis can be altered in degree and for that sort of information, one has to turn to animal experiments.

Colin Adams has studied regression of atherosclerosis in rabbits and, by producing weight loss with a low-calorie diet, he has caused considerable inanition but there has been no detectable degree of regression of previously induced atherosclerotic lesions.

<**Lewis over slide showing atherosclerosis in rhesus monkeys before and after a cholesterol-reducing diet, then to camera**>

On the other hand in the rhesus monkey, there is fairly good evidence of regression from the work of Armstrong and Connor in Iowa City. And what they have done is to induce, over a period of 10 to12 months, this sort of gross atherosclerosis in rhesus monkeys by feeding them on a diet such as we, in Western societies, habitually eat. Now, he's sacrificed one group of monkeys after 10 or 12 months on that diet, and has then taken the remaining group of animals and put them on a cholesterol-reducing diet, one rich in polyunsaturated fat and poor in cholesterol, and followed these animals for a period of another 40 months, in fact, almost 4 years. And in the lower frames you can see the sort of appearance that can be attained in these regression animals after 3 to 4 years on a regression diet, so it may be that there are some species differences in their ability to produce regression.

The evidence in man is really quite limited. There is one small group of hyperlipidaemias of genetic origin, which are very susceptible to treatment. And there is a study by Zellis and Levy in which such patients who had peripheral vascular disease have been shown to produce considerable increase in peripheral blood flow after 6 months on a lipid-lowering diet and drugs.

Colin Adams will now discuss some of the theoretical aspects of regression studies in atherosclerosis.

<**Adams, shown seated, to camera, then over diagram showing how cholesterol can be removed from the arterial wall, then to camera**>

If there is regression of atherosclerosis, from what Barry Lewis has been saying, it is a rather slow process; it's not at thing that you would expect to see in a matter of months, rather it is a matter of years. And if we now look at this diagram here, this may help to explain some of the difficulties in getting rid of cholesterol from the arterial wall. The cholesterol that has got into the arterial wall has come down to lipoprotein; if it stays as lipoprotein, it will be water-soluble. However, it would seem

that much of this cholesterol on the lipoprotein is split off to form an inert metabolic pool that's not easy for the tissues to handle. If it were in the liver, of course, it could break it down, as I said before, to bile salts. But there's no way that the arterial wall can handle and get rid of crystalline cholesterol, except possibly by phagocytosis. But when we look at phagocytosis, smooth muscle does not seem really to take up any significant amount of the crystalline cholesterol. There are very scanty and very few typical macrophages in the arterial wall and perhaps it's this relative absence of macrophages which is an important factor in the inertia of the atheroma lipids. For example, if one takes a sample of atheroma lipids and injects it under the skin in the rat, those lipids go in 12 weeks. In the arterial wall it would seem to take a matter of years. And in the skin there are plenty of macrophages, in the arterial wall there are few, if any.

And maybe the answer lies with Bernard Shaw, in 1909, who said that there is only one treatment in medicine, that is, to stimulate the phagocytes. Perhaps he was right. But I know that Neville Woolf feels that the position is much more complicated.

<**Camera pulls back to show Woolf, Lewis and Adams seated around a table**>

<**Woolf to camera**>

I should like to sound a slight note of warning here and that is that we should be careful before we equate changes in the incidence of clinical events with changes in vessel wall disease. There are not many studies, but in one, which shows a sharply declining mortality from coronary heart disease in physicians who have stopped smoking, the change in mortality rates took place so fast that I can't credit that this is due to any major change in vessel wall disease. And though, of course, in communities with very little atherosclerosis, there is equally very little in the way of clinical arterial disease, we must look also to the complications, and in particular the thrombotic complications, which are associated with atherosclerosis, whether causally or no, if we are to find an answer to this plague.

<**End credits**>