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## CORONARY ARTERY DISEASE\*

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[WITH SPECIAL PLATE]

When I could no longer hide the title of this lecture from members of my laboratory one of my colleagues remarked bluntly, "You have been foolhardy." This is unfortunately true, and the force of the remark was further borne in on me when I read an admirable review entitled *Lipids in Relation to Arterial Disease* by Hilditch and Jaspersen (1959). They said: "Faced with a problem of such extreme complexity—involving possibly not only dietary fat but also protein, and into which perhaps endocrine metabolism as well as enzyme metabolism and their possible disorders, and other factors such as sex, heredity, nervous strain, sedentary or mobile occupations, and so on may enter—it seems to us both unfortunate and surprising that some medical investigators have been rather prone to seize on one or other facet of the problem and thereon to formulate hypotheses incongruous with the slender weight of the experimental findings on which they are based."

I will try to set out as clearly as I can some of the pathological changes involved in coronary artery disease, to mention some of the theories on their origin, and then lead on to discuss how more progress might be made in the examination of the changes in a very common disease which is often associated with crippling effects on the cardiac muscle or sudden death. Therapeutic measures will also be mentioned.

### Components of Coronary Disease

There are two separate but probably associated components of coronary artery disease—namely, alterations in the wall of the artery itself and alterations in the content of the artery.

First let us consider the wall of the artery. Electron microscopy makes it possible to obtain a clearer idea of the intima of the coronary artery, in which it is supposed the initial lesions of atherosclerosis occur. It has so far not proved possible to obtain good human material, but there is little reason for supposing that much difference exists between human and pig arterial tissue. The figure was obtained from the pig. It shows that the artery is lined by a single layer of endothelial cells, which at this magnification are structures of considerable size, and that these together form a continuous cellular barrier between the lumen of the artery and the tissue of the intima. There is scarcely any space between the cells at their junctions, certainly not a substantial amount of cement as is still commonly believed to exist between endothelial cells.

How substances are transported through this cellular barrier is a problem common to all endothelium, and

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it has not yet been solved. The properties of endothelium deserve more attention than is usually accorded to them, for through this layer of cells all nutrients and fluids reaching the intima and immediately subjacent media have to pass. Below the endothelium is a layer in which collagen, elastic tissue, and some other fine fibres can be seen. They are probably embedded in a mucoprotein matrix which does not show in electron micrographs. Outside this layer the intima is bounded by a fenestrated elastic tissue membrane. (No fenestrae are shown in the Special Plate.) In the fenestrae smooth muscle cells, such as are present in the media, sometimes occur. It is quite possible that the scattered cells normally encountered in the intima are smooth muscle cells and not fibroblasts or macrophages.

### Developed Lesions in Man

Before attempting to inquire what are the initial changes of atheroma it may be profitable to consider what can be present in fully developed lesions. The constituent of the intimal thickening or plaque which has attracted most attention is lipid. This material may be free in the tissues or inside cells which are usually considered to be macrophages but which could be smooth-muscle cells. Lipid may also be found in the neighbouring media. At the base of the intimal plaque a semi-fluid mass of lipid and cholesterol crystals may be present. It is this which has given the name atheroma to the lesion. Considerable amounts of calcium may be encountered as well as fibres usually regarded as collagenous and elastic in nature. Eventually there may be vascularization, ulceration, and haemorrhage into the plaque. The intimal thickenings may be of great variety, eccentric or fairly evenly spread around the lumen; the fibrous component may be most conspicuous in some lesions but in others the lipid. The central problem is to explain why these constituents appear at discrete points in the coronary arteries.

It has been known for over a century that there is a considerable amount of cholesterol in the lipid that accumulates in atherosclerotic lesions. Indeed, the public is now aware of cholesterol and its putative connexion with the development of atherosclerosis. For example, there was, not long ago, a large advertisement in *The Times* asking, "How's your cholesterol?" and urging us to eat more fish to lower its level; and, again, a very penetrating cartoon in the *New Yorker* in which a woman at a cocktail party inquires, "Are you a no-cholesterol doctor or are you one of those no-cholesterol-is-all-bosh doctors?" This cartoon represents fairly the confusion that exists about the relationship of lipids to the genesis and development of atherosclerosis.

### Evidence Other than Morbid Anatomical for the Involvement of Lipids

What are the reasons, beside the morbid anatomical findings, to connect errors of lipid metabolism with atherosclerosis?

There is considerable evidence of some relationship between the amount of lipid in the blood, the severity of atheromatous lesions, and the incidence of coronary thrombosis. Thus it has been found that some races which have a low incidence of coronary thrombosis live on diets with a low fat content. They have a low level of cholesterol in the blood, and a cholesterol/phospholipid ratio of about one. There is also a relatively low concentration of  $\beta$ -lipoprotein. Many patients who have coronary heart disease have a higher mean cholesterol level in their serum than so-called normals, a high concentration of  $\beta$ -lipoproteins, and a cholesterol/phospholipid ratio greater than one. It is generally agreed that patients with diabetes mellitus, myxoedema, and familial hypercholesterolaemia, in whom raised serum cholesterol levels and other abnormalities of the lipid occur, have a high incidence of atherosclerosis. Nevertheless, many patients with atherosclerosis do not show such abnormalities of serum lipids.

Interest is now moving to a detailed comparison of the composition of serum lipids with that of the lipids occurring in plaques, a comparison made possible by spectacular improvements in methods of chemical analysis. Most of the work has been done on aortic material. It may well be that the lipid content of coronary lesions and those of the aorta are similar, but they are not identical. For example, it was recently shown that triglycerides formed 50% or more of the total lipids in some lesions of the coronary arteries, which is very much more than in aortic lesions, while cholesterol esters and free cholesterol were proportionately less.

So far as aortic lesions are concerned the percentage of total lipids in the lesions increased with increasing severity of the lesions, and cholesterol and its esters increased more markedly than the phospholipids (Böttcher, Keppler, Romeny-Wachter, Boelsma-van Houte, and Van Gent, 1958; Böttcher, Woodford, Romeny, Boelsma, and Van Gent, 1959). In view of the importance that has been attributed to polyunsaturated fatty acids such as linoleic and arachidonic acids it is well to note that at all stages the cholesterol ester fatty acids were mainly unsaturated, oleic acid constituting about 40% and linoleic acid 25% of the total fatty acids. With increasing severity of the lesions the proportion of unsaturated fatty acids in this fraction actually increased.

The lipids contained in atherosclerotic plaques are apparently qualitatively the same as those circulating in the blood. Hence a very commonly accepted view of the formation of an atherosclerotic patch is as follows.

Lipids circulate in the plasma mainly in the form of  $\alpha$ - and  $\beta$ -lipoproteins. The  $\alpha$ -lipoproteins are maintained at a relatively steady level in the blood, and it is the  $\beta$ -lipoproteins, which have the larger molecule, that are increased in amount when the cholesterol level of the blood is raised. The  $\beta$ -lipoproteins are rather ill-defined substances, composed of protein, cholesterol, phospholipids, and fatty acids. It is supposed that in the atheromatous artery the  $\beta$ -lipoproteins in the blood pass through the endothelial layer in greater amount than

normal. Our ignorance of the mechanism by which substances pass through endothelium is profound, but the mechanism could be of great importance in connexion with this theory of atherogenesis. At all events, it is supposed that lipid passes in some way through the endothelium and reaches the subendothelial space, which is shown, highly magnified, in the Special Plate. In this space something happens so that the rather unstable lipoproteins are broken down, with the liberation of cholesterol, fatty acids, and phospholipids. In this connexion a recent observation of Kayahan (1959) may be pertinent. In investigating the excess fat in atherosclerotic intimas he obtained evidence that globulins from atherosclerotic intimas could bind considerably more lipid than globulins from normal intimas.

Some fatty acids, phospholipids, and protein pass on through the tissue spaces of the media into the lymph vessels in the adventitia and so are removed, but cholesterol, both as free cholesterol and as esters, is relatively insoluble and so tends to stop in the intima. Thus cholesterol and a certain amount of phospholipid are left behind. Compared with the composition of serum lipids the phospholipid in the plaque is less in proportion to the cholesterol; in other words, the phospholipid/cholesterol ratio is lower. Under this theory of the mechanism of atherogenesis this insoluble residue is the initial lesion. With the passage of time it increases in amount, macrophages appear, fibrous tissue develops, and so on, until plaques containing much lipid and fibrous material are built up. Under this theory the lipid is directly derived from the plasma. The calcium salts which are often prominent must also come from the same source.

What I have just outlined is a relatively simple theory associating the content of cholesterol and other lipids in the blood with the accumulation in the arterial wall. Such a view is plausible, and indeed many pathologists would say that there was evidence that the earliest lesion in the formation of an atherosclerotic plaque was a "fatty streak" or a collection of lipid just beneath the endothelium. On the other hand, there are pathologists who maintain that the so-called fatty streaks which can be seen in the aortas of even very young people have nothing to do with the development of the serious lesion of later life.

Great attention has been given to dietary lipids in the belief that these substances may in some way determine the deposition of fats in the intima of arteries, but it should be mentioned that some observers think that the protein content of the diet is of great importance (possibly as important as the lipid content) in controlling serum lipid levels (Kokatnur and Kummerow, 1959). If this should be true, clearly some present-day views on the atherogenic properties of "western" diet would have to be considerably recast.

There is also the possibility that the lipid is formed by the activity of cells in the vessel wall. Phospholipid can certainly be synthesized there, and a recent note on an examination of human aortic and coronary lesions with the electron microscope states boldly that the fat is intracellular and is found especially in smooth muscle. The suggestion is made that the accumulation of fat in atherosclerotic lesions is the result of metabolic processes in the cells of the arterial wall (Geer, McGill, Strong, and Holman, 1960).

I have briefly mentioned only a small fraction of the very large amount of work on the association of the

lipid content of the blood and atherosclerosis, but even if I had time to review more evidence I should still say that on the whole the observations do not carry complete conviction that an increased level of blood lipids is the primary cause of atherosclerosis, though it may well be important in the development of the lesions.

#### Elastic Tissue

But there are other structural changes in atherosclerosis besides the accumulation of lipids.

A number of workers have demonstrated that from birth onwards progressive changes can be shown in the elastic tissue of the intima of the coronary arteries. At birth the internal elastic lamina appears in a section as a smooth corrugated continuous membrane, but even during infancy this tends to fragment and to reduplicate, so that by the end of the second decade the intima may have thickenings in which elastic tissue is prominent. This is so usual that it must be considered to be a normal part of development.

It is conceivable that the mechanical stresses of the circulation, especially at branchings of the vessels, may put a constant strain on arterial tissue, especially the elastic fibres. At junctions, eddies in the blood-stream are likely to form, and these eddies may set up high-frequency vibration which might produce obvious damage after many years. It is certainly true that lipid deposits can often be seen first in relation to elastic-tissue fibres.

It could be that mechanical damage to the arteries might in this way initiate lesions, and this should be very seriously considered, for the patchy distribution of atherosclerosis must be explained. There is a possibility that mechanical damage to the arterial wall may favour the deposition of lipid from the blood. Colour is lent to this supposition by the experiments of Waters (1957), who made use of the fact that he could damage the walls of the coronary arteries of dogs by the administration of allylamine. If lipoprotein in considerable amounts was injected into the blood-stream lipid accumulated at the sites of damage in the coronaries, and the structure of resulting lesions bore some resemblance to the human lesion.

#### Ground Substance

There is one other constituent of the intima which we have not considered, and that is the ground substance which stains metachromatically with such dyes as toluidine blue. Through this ground substance, which probably contains mucopolysaccharide, everything which leaves the lumen of the vessel has to pass. There is no reason why the primary lesion should not be due to some change in it, but the evidence for such a change is very small. It can be said, however, that in well-developed atherosclerotic lesions the amount of stainable ground substance may be increased.

#### Changes in Blood

We have so far considered changes occurring in the wall of the coronary artery. What about changes in the blood circulating in the artery?

There is sometimes confusion between clotting and thrombosis. Thrombosis, according to current information, is initiated by the deposition of platelets on the wall of a vessel in which blood is flowing—probably a damaged wall. These deposits build up, entangle white

corpuscles, and entrap red cells. With growth of the thrombus fibrin appears, but this is not an initial constituent. Thus a thrombus is an intravascular object with a characteristic structure, whereas a clot is formed by fibrin, which entangles the blood constituents in its immediate vicinity. Clots similar to those forming *in vitro* may form in blood made stagnant by the blocking of a vessel with a thrombus, and so they may be of importance, though secondary, in coronary thrombosis. The mention of coronary thrombi usually conjures up a picture of the final complete blocking of the affected artery, but Duguid (1946, 1948, 1949) and his colleagues have produced weighty evidence in favour of a theory, put forward by Rokitsansky in the eighteenth century, to the effect that at an early stage the lesion of atheroma is due to the deposition of fibrin on the inside of a vessel wall. Subsequently the deposit becomes incorporated in the wall.

Few would, I think, now deny that the organization of mural thrombi or deposits of fibrin plays an important part in the enlargement of atherosclerotic plaques on which they occur, but many are not convinced that mural thrombosis or deposition of fibrin explains the initial lesions. It is, for example, impossible to derive the amount of lipid present in plaques from breakdown products of red cells or other constituents contained in a thrombus of suitable size. But even if we only agree that mural thrombosis adds to lesions already existing it becomes a very important process, and our attention is concentrated not only on the state of the wall but on the state of the blood circulating in the vessel.

Epidemiological studies by Morris (1951), combined with the examination of the records of necropsies carefully conducted many years ago, indicate that it is an increase of thrombosis and cardiac infarction which is the feature of the present time and not an increase in atherosclerosis. If this is indeed the case you will note that such a view appears to be incompatible with Duguid's theory, for if more thrombosis is occurring to-day than formerly and mural thrombosis is a cause of atherosclerosis, then atherosclerosis should be much more frequent to-day than it was. This does not appear to be so.

Many attempts have been made to show that the blood of atherosclerotics clots more easily than that of so-called normals. Even if this were so it would not show that it was more apt to deposit a thrombus in a vessel, for this depends on alterations to platelets. In this connexion McDonald (1960) has found that patients suffering from ischaemic heart disease have abnormally sticky platelets as judged by an *in vitro* test. This work needs to be confirmed and extended, and the relationship between platelet stickiness *in vitro* and a tendency to thrombosis in the body requires further exploration before such findings can be definitely related to disease in man.

The relationship between lipids and blood-clotting has been investigated extensively, but there has been much controversy over many of the findings, and comparatively few facts are established in such a manner as to command general agreement. At the present time not enough is known about the connexion between blood coagulation in a test-tube and thrombosis in the body to assess the likelihood that these findings are of pathological significance.

If fibrin formation is important in human arterial disease, not only clotting but the converse process of

fibrinolysis may be important. It has been known for a long time that blood may develop fibrinolytic activity after injury, severe exercise, or emotional stress. Eight years ago it was discovered that all human blood shows a labile fibrinolytic system, provided that the blood is rapidly cooled after collection (Fearnley, Revill, and Tweed, 1952; Truelove, 1953; Fearnley and Tweed, 1953), and it has been claimed that this labile fibrinolytic system is partly inhibited after a fatty meal. Recently this claim has been denied (Greig, 1956; Greig and Runde, 1957; Nitzberg, Peyman, Goldstein, and Proger, 1959; Billimoria, Drysdale, James, and Maclagan, 1959; Hougie and Ayers, 1960). The methods used by the different investigators were not the same, so the apparent confusion may turn out to be methodological. At present, however, the position is far from clear.

The belief that atheromatous plaques may be derived from thrombi has naturally led to attempts to demonstrate fibrin in them. Here conventional staining methods are not of much use. Levene (1955) showed that atheromatous plaques contained a fibrous protein whose appearance in the electron microscope resembled that of fibrin. Crawford (1960) and his colleagues have attacked the problem by using fluorescent anti-fibrin antibodies and have been able to show specific fluorescence in plaques. Indeed, Crawford and Wolff (1960) showed such fluorescence in aortic intimal fatty streaks. Possibly fibrin might be formed in plaques *post mortem*, for the endothelium rather quickly disintegrates and plasma might seep in and clot in the intima. In short, the evidence that atheromatous plaques contain fibrin is suggestive but not as yet conclusive.

#### Animal Experiment

Can we learn anything more definite about the origin and development of atherosclerosis by animal experiment? One always hopes by such means to isolate the essential factors in a problem and so simplify the understanding of the phenomena involved.

Some 50 years ago Anitschkow and Chalataw discovered that if rabbits were fed a diet containing considerable amounts of cholesterol they developed lesions in the intima of their aortae which bore some resemblance—they thought a close one—to those occurring in man. The level of cholesterol in the serum of rabbits may rise to as much as 1,000 mg. per 100 ml. or even more.

How does the lipid material which is thus in great excess in the blood gain access to the intima of the vessel? I think it is clear in some of this rabbit material that the endothelial cells over well-developed plaques, but not elsewhere, contain considerable amounts of sudanophil substance in their cytoplasm, but it cannot be said whether this is lipid in transport to the intima (Poole and Florey, 1958).

If the lipid does not pass directly through endothelium, are there other possibilities?

One of the features of the rabbit lesion is the presence of large numbers of lipid-laden macrophages. Macrophages filled with lipid can be found in passage through the endothelium, but of course it is not at all certain in which direction they are going; they might be going either outwards or inwards. But macrophages containing lipid have been demonstrated in the circulating blood of cholesterol-fed rabbits by Rannie (1959), and many observers have seen that macrophages may be present on the endothelial surfaces of such plaques.

Poole and I believe that if the macrophages which come to rest on the surface go into the plaque they do so by penetrating the endothelium, as Leary (1941), who considered that the macrophages arise in the liver, was the first to suggest. Rannie and Duguid (1953), on the other hand, believe that the macrophages adhere to the surface and endothelium then grows over them, and that thus the macrophages are incorporated in the plaque.

There is no evidence, so far as I am aware, that macrophages transport lipid in human atherosclerosis in the way postulated for the rabbit lesion. Moreover, in the cholesterol-fed rabbit there is a large deposit of cholesterol in the liver and spleen and in phagocytic cells generally throughout the body. The rabbit disease is, in fact, a cholesterosis. When cholesterol feeding is continued for some time the lesions produced in the rabbit's aorta resemble those which are found in man in that they contain fibrous tissue and free lipid in the base of the lesion.

By feeding cholesterol in large amounts similar lesions can be produced in chickens, and a great deal of work has been devoted to studying the influences which affect the deposition of this cholesterol. It has also been found possible to produce lesions in dogs, monkeys, and rats, but cholesterol feeding alone is insufficient. Auxiliary methods to raise the level of blood cholesterol have to be employed.

None of the experimental lesions mentioned are accompanied in the ordinary course of events by ulceration, haemorrhage, or thrombosis. Suggestive as these experiments are, it must be confessed that they leave much to be desired. Nevertheless, there is no doubt that they have been taken to strengthen the view that a high blood cholesterol is responsible for the genesis of human atherosclerosis.

#### Naturally Occurring Atherosclerosis in Animals

Recently, increasing interest has been taken in naturally occurring atherosclerosis in species other than man. For example, some very interesting observations have been made on pigeons by Clarkson, Prichard, Netsky, and Lofland (1959), who discovered that certain breeds regularly developed lesions in their aortae, and that the lesions bore a very close resemblance to those in man. They sometimes broke down, with the production of a porridge-like material as in human lesions. They sometimes ulcerated, with thrombus formation on the surface.

This condition is particularly important in that it occurs in the absence of special dietary procedures, and only in certain breeds of pigeon. Other breeds do not show it when kept under comparable conditions. In other words, there seems to be a very strong genetic element in the development of this particular atherosclerotic lesion. The pigeons, whether they bore lesions or not, had the relatively high blood cholesterol of, for example, around 350 mg. per 100 ml. (Lofland and Clarkson, 1959).

The baboon has also attracted attention. Lindsay and Chaikoff (1957) found lesions in two zoo baboons, and Gillman and Gilbert (1957) described lesions resembling those in man in the aortae of baboons in the laboratory. The lesions developed in the presence of low concentrations of cholesterol in the blood, and indeed this level sank with age. It has since been discovered that baboons living in the natural state in Kenya, and probably on a fat-poor diet, occasionally

develop fatty streaks, and, more rarely, fibrous plaques in the aortae (McGill, Strong, Holman, and Werthessen, 1960). At present baboons are being examined in a colony which is being raised in Texas. So far nothing new about the causation of the initial atherosclerotic lesion has emerged from these studies, but clearly the baboon, although intrinsically difficult to deal with, may have possibilities for future research.

Farm animals, and perhaps also those in the wild, seldom survive to biological old age, and little is known about the frequency of such changes in old animals. Recently Jennings, Stehbens, French, and I have had an opportunity to examine the vessels of a pig which reached the mature age of 7 years and 4 months, which is slightly past middle life for a pig (Burrow, personal communication). This pig had an isolated pyloric pouch which had secreted mucoid juice for seven years, but there is no reason to connect the arterial lesions with the events in the pylorus. At post-mortem examination the aorta and coronary arteries showed atherosclerotic lesions which bore a striking resemblance to partly developed lesions in man, and there were elastic-tissue changes in the coronary and cerebral vessels similar to those which occur with advancing age in man. Of particular interest is the fact that plaques and diffuse fat-containing intimal thickenings were present in the first few centimetres of the coronary arteries before they entered the muscle; many of the plaques were at bifurcations. Elastic and collagenous tissue, smooth-muscle fibres, and deposits of fat and of calcium were present in the coronary and aortic plaques, though there was no definite softening and no ulceration, haemorrhage, or vascularization (Jennings, Florey, Stehbens, and French, 1961).

It might not be going too far to believe that the same factors were operating to produce the lesions in the pig as are present in man. For at least the last three years of its life the pig was on a fat-poor diet.

There are in the literature some other indications that the pig may be a suitable animal for experiment because naturally occurring aortic plaques, albeit small, increase in frequency with age even in quite young pigs. Commercial pigs seldom live to be much more than 3 years old, but lesions were found in over 30% in the oldest group of a series obtained from the slaughterhouse (Gottlieb and Lulich, 1954). An increase of lesions has been described in young pigs after feeding with butter, though others have not found this effect (Rowell, Downie, and Mustard, 1958).

The pig, like man, is omnivorous, and it is said to have a similar cardiovascular system and metabolism to that of man—whatever that may mean precisely—and the composition of the plasma lipoproteins has been said to be in some respects more like that of man than of other mammals. Different strains of pig may show consistent differences in the concentrations of serum lipids and cholesterol (Lewis and Page, 1956), so that it might be possible to choose especially suitable strains for particular purposes. I will be rash enough to suggest that in the pig we have an animal with which we might solve many of the outstanding problems of atherosclerosis. To do so would require extensive effort. It would first be necessary to demonstrate that a substantial proportion of ageing pigs show the lesion. If this should prove to be the case it would not be impossible to plan experiments which would test the many theories concerning the genesis of atherosclerosis and to evaluate critically the

effects of therapy, whether they be of a dietary or other nature. I stress that ageing pigs should be used. It is perhaps worth noting here that the pigeons I mentioned previously were 7 years old.

#### Prevention and Treatment

The study of disease processes in man or animals not only has its intrinsic interest, but holds out the possibility that its results may be applied in human disease. So one might inquire whether the enormous number of observations and experiments already recorded have given us anything firm on which to base prevention and treatment.

If one considers that the origin of atherosclerosis is due to errors of lipid metabolism with the presence of a high cholesterol level in serum, then dietary manipulation may be of great importance. How does one set about lowering the blood cholesterol? It has been shown that it is possible to lower blood cholesterol and the  $\beta$ -lipoprotein level both in man and in animals by the administration of certain hormones such as oestrogens and thyroid hormones, but so far their use for this purpose has not been clearly shown to be beneficial to patients with atherosclerosis.

Of recent years it has been demonstrated by a number of workers that relatively high levels of cholesterol in the blood of man can be lowered by feeding substantial amounts of fats containing various polyunsaturated acids, such as linoleic and arachidonic and other acids contained in menhaden oil (Ahrens *et al.*, 1959). Why does the level of blood cholesterol sink? Is the cholesterol metabolized and got rid of from the body in some form, or is it merely removed from the blood and stored somewhere, perhaps even in the arterial wall, or is there a diminution of the endogenous production of cholesterol? There is some evidence from patients with biliary fistulae that excretion in the bile of cholic acid, which can be derived from cholesterol, is greatly increased after the ingestion of sunflower-seed oil—an oil rich in polyunsaturated fatty acids—or after the intravenous injection of cottonseed oil (Gordon, Lewis, Eales, and Brock, 1957; Lewis, 1958), and there is independent evidence that faecal excretion of cholesterol and its transformation products is increased after the administration of certain dietary fats containing polyunsaturated fatty acids. Thus the unsaturated fatty acids may accelerate the catabolism of cholesterol to cholic acid.

It is, however, by no means certain that lowering the level of serum lipid by the administration of polyunsaturated fatty acids has any effect on the development of atherosclerosis or on the incidence of thrombosis, and we badly need well-planned observations on a very considerable scale to settle this point.

To mobilize the lipid in plaques already fully formed seems to be an even more formidable undertaking, but perhaps it is not impossible, for recent results on natural pigeon atherosclerosis suggest that birds given polyunsaturated fatty acids in their diet for a year have less extensive lesions than pigeons not so treated (Clarkson and Loffland, 1960).

The idea that the lipid in a plaque in a coronary artery might be at least partly removed is made more hopeful by the fact that 50% may be triglyceride, and it has been shown that triglyceride is readily oxidized by macrophages (Day and Harris, 1960; Day, 1960).

Perhaps some benefit might ensue by merely preventing the accumulation of new triglyceride. Whether the fibrous material would get less is problematical—indeed, the whole proposition is problematical.

If one takes the view that mural thrombosis is all-important, clearly steps to prevent thrombus formation are of the greatest interest. A thrombus begins by the deposition of platelets around which white cells and fibrin collect. It is commonly assumed that an anticoagulant such as heparin will prevent thrombus formation, but this is not necessarily true, for Poole (1959) has found that in an *in vitro* system heparin does not affect the agglutination of platelets to form thrombi although fibrin formation is stopped. Possibly some substance which prevents platelet agglutination might be found helpful in controlling the early stages of the disease, but a substance with this property which would be harmless to the body does not, so far as I am aware, exist.

Since fibrin forms part of a thrombus it is not unreasonable to suppose that anticoagulants might be helpful in treating coronary thrombosis by preventing extension of the thrombus, but I understand that there is at present no agreement about the advantages of giving prolonged anticoagulant treatment to those who have had a coronary infarction. Other possibilities exist—for example, plasmin might be used to remove the fibrin component of a thrombus and so open up an artery again—but so far nothing practicable for human therapeutics has emerged.

### Surgery

It has been said that surgeons score some of their greatest successes when unblocking tubes. A few years ago it would have sounded fantastic to suggest that it might be possible to remove material surgically from the lumen of a coronary artery, but such a procedure is now carried out. Endarterectomy is often successful in restoring circulation in large arteries such as the aorta, but smaller vessels tend to thrombose again. The operation is in what the surgeons call the experimental stage, but their efforts will be watched with great interest, and it is quite possible that future treatment of advanced coronary disease may lie with them.

Less hazardous than endarterectomy is the attempt to improve collateral circulation by putting an irritant into the pericardial sac. Small vessels form in the resulting inflammatory tissue, and at one time it was believed that they enabled blood to pass from the parietal pericardium into the heart, but now it is suggested that the new vessels only offer new anastomotic channels between the coronary arteries themselves and so facilitate a more even distribution of the blood reaching the heart.

More radical procedures to bring new blood to an ischaemic myocardium involve grafting external structures into the heart or on to its surface. Of these, perhaps the most interesting is transplantation of the mammary artery. The originator of this procedure (Vineberg, 1946, 1954; Vineberg and Miller, 1953; Vineberg, Munro, Cohen, and Buller, 1955) implanted an open mammary artery into the ventricular muscle and thought that the blood issuing from the vessel would find its way through spaces in the muscle to the ventricular chamber, and that the artery would sprout and eventually join up with the coronary arteries and so increase the blood supply to the heart. Injection of

such transplanted arteries with plastic substances shows that they do eventually form connexions with the coronaries, but it does not seem at all probable that the mammary artery sprouts fully differentiated arteries which join up with ventricular arteries. Some light has been thrown on the possible mechanism involved in the development of the additional blood supply by examining the march of events in other tissues. When a piece of duodenum is implanted subcutaneously in a rabbit, vessels from the surrounding tissue unite with vessels of the gut, but this union is accomplished by the sprouting of numerous small vessels, probably of capillary size, which join up and are then modelled to form arteries and veins which connect with the original arteries and veins (North, Sanders, and Florey, 1960).

From some experiments on implantation of mammary arteries of the pig into the ventricle we think that the vessel soon thromboses near its free end and that then small vessels, possibly from the vasa vasorum, sprout and connect with pre-existing ventricular vessels and eventually enlarge to form arteriolar connexions. After operation in man the connexions which develop between the mammary artery and the coronaries do not appear to carry a very substantial amount of blood, and this operation has not gained much currency among cardiac surgeons.

I have already taxed your patience by presenting so much that must be common knowledge to all, but I would like finally to point out the enormous change in point of view which has taken place in the last 30 or so years. Formerly atherosclerosis was thought to be a manifestation of old age about which nothing could be done. No doubt changes that we must consider to be inevitable with advancing years do play a great part, much as we may dislike that idea, but the increased incidence of coronary artery disease in young people has greatly stimulated the examination of all aspects of the disease. We are still in a state of much confusion, but if I have done anything to indicate some of the growing points of investigation I shall be very happy.

[This lecture was illustrated by many slides. It has in part been rewritten to avoid reference to them. My thanks are particularly due to Dr. M. A. Jennings and Dr. J. C. F. Poole for their assistance in preparing the lecture.]

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## IMMUNOLOGICAL DIFFERENCES BETWEEN NORMAL AND MALIGNANT CELLS

BY

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[WITH SPECIAL PLATE]

One of the traditional concepts of malignant change is that neoplastic cells lack certain growth-controlling enzyme systems present in normal cells. On this hypothesis, normal cells should contain proteins which are absent from malignant cells, and this difference in constitution should be demonstrable by immunological methods. Such an approach has been made by Weiler (1952), who compared the immunological properties of cytoplasmic particles from rat liver and from primary hepatoma induced by 4-dimethylaminoazobenzene (D.A.B.). He described an organ-specific antigen present in normal liver cells, but apparently absent from tumour. By means of the fluorescent antibody method he showed (1956a) that the livers of D.A.B.-treated rats developed islands of cells in which the antigen concentration was reduced in comparison with the surrounding parenchyma—that is, there was diminished staining of these islands in histological sections treated with specific anti-liver serum conjugated with fluorescein. The degree of antigen impoverishment varied with the total dose of carcinogen and the duration of the experiment. These findings indicated a correlation between loss of antigen and carcinogenic change. Similar observations were made in subsequent studies (1956b, 1956c) of stilboestrol-induced carcinoma in hamsters. The work, which has been recently reviewed (Weiler,

1959), has not, so far as we are aware, been confirmed by other workers, though an unsuccessful attempt to repeat it is reported by Hughes *et al.* (1957).

In the present investigation into the antigenic differences between normal tissues and tumours derived from them Weiler's findings in the D.A.B.-induced rat hepatoma have been corroborated. Similar results have also been obtained in preliminary studies of carcinoma in hamster kidney and of human skin tumours.

### Methods

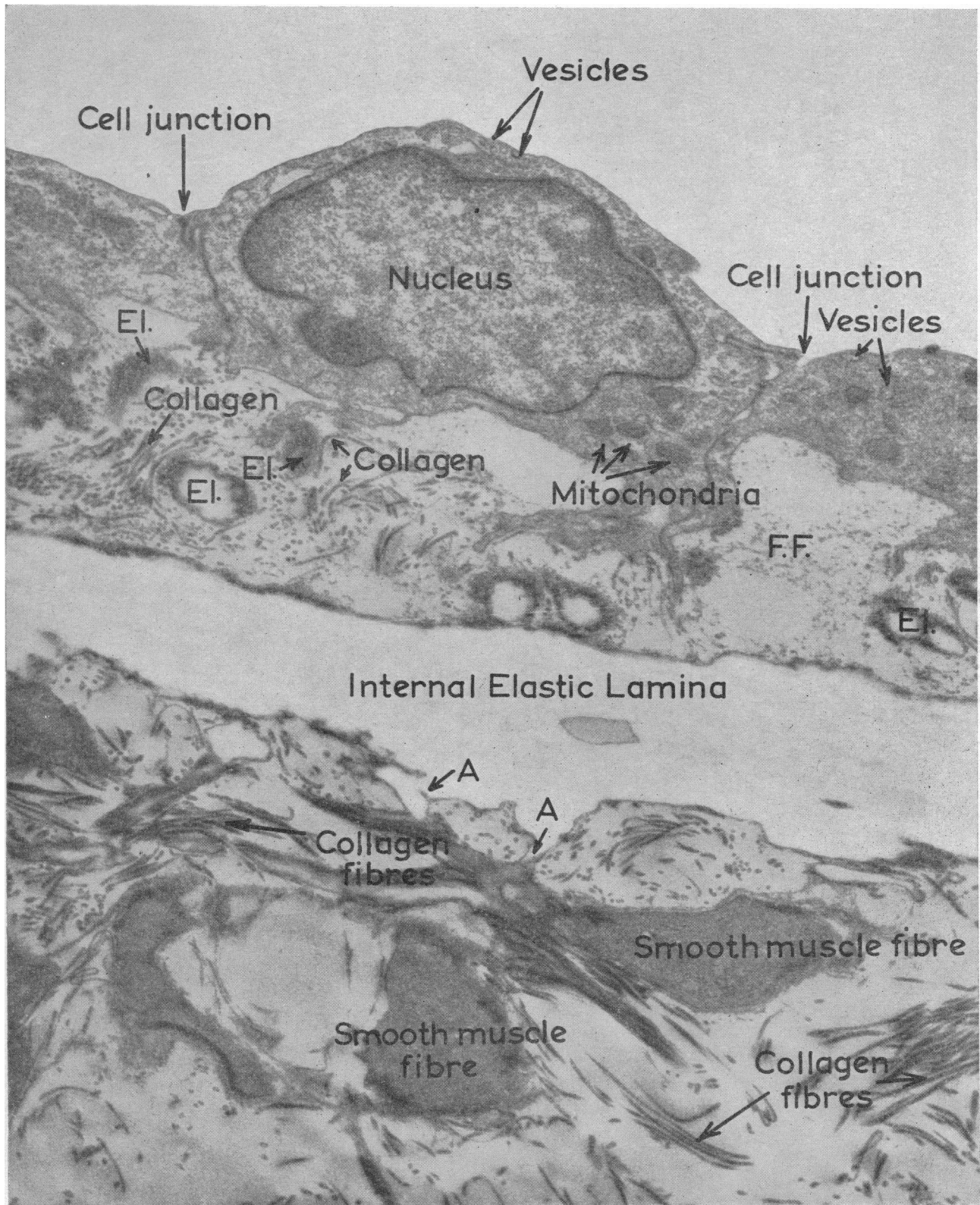
#### Liver Carcinoma in Rats

**Production of Tumours.**—The method was based on that of Griffin *et al.* (1948). Male albino rats (Tuck strain), weighing 200–250 g., were fed *ad lib.* for six months on a low-protein diet containing 0.06% 4-dimethylaminoazobenzene or for ten weeks on the low-protein diet containing 0.08% 3'-methyl-4-dimethylaminoazobenzene. When liver tumours became palpable the rats were killed by ether anaesthesia; thin blocks, each containing normal and tumour tissue, were "snap-frozen" in screw-cap bottles, pre-cooled in alcohol dry-ice freezing mixture, and stored at  $-20^{\circ}$  C. Parallel blocks of tissue taken for conventional histological examination were fixed in formol-corrosive.

**Preparation of Antigen.**—Finely chopped fresh rat liver was suspended (10% w/v) in chilled 0.25 M sucrose buffered at pH 7.3 (0.1 M phosphate). The suspension was homogenized for four minutes at about 10,000 r.p.m. in an M.S.E. homogenizer. Further cellular disintegration, controlled by microscopical examination, was accomplished by means of the M.S.E.-Mullard ultrasonic disintegrator. A total of 90 seconds sonic treatment was given, resulting in breakdown of about 80% of cells with dispersal of cytoplasmic components; the majority of the nuclei remained intact. The cell components were separated as described by Weiler (1956a): 10 parts of the homogenate were layered on to one part of 10% sucrose solution, and centrifugation was carried out at 1,750 g for 7 minutes to remove tissue fragments and cell nuclei. The supernatant was then centrifuged at 9,000 g for 10 minutes to remove the mitochondrial sediment. Further centrifugation at 25,000 g for 90 minutes yielded cytoplasmic particles derived from the endoplasmic reticulum—that is, microsomal material, but presumably contaminated by fragments of cell membranes, nuclei, and mitochondria. This sediment was washed by resuspending in buffered sucrose followed by centrifugation, and the final sediment was snap-frozen in alcohol dry-ice freezing mixture and stored at  $-20^{\circ}$  C. Weiler (1956a) showed that this final fraction provoked the best organ-specific antiserum, and it was therefore used as antigen throughout the following experiments.

**Production, Absorption, and Testing of Antiserum.**—The antigen was suspended in buffered saline (0.01 M phosphate, pH 7.1) at a concentration of 3.75 mg. antigen nitrogen per ml., and administered to three 2.5-kg. cross-bred female rabbits in 0.5-ml. doses intravenously at two- or three-day intervals for 24 days; the total dose of antigen N given to each rabbit was 18–19 mg. The animals were bled six days after the last injection. One rabbit produced a good antiserum with a complement-fixing titre of 1/320, and this was used in subsequent experiments; the titres in the other two rabbits were only 1/80 and 1/40 respectively. Complement-fixation tests were carried out with serial doubling

SIR HOWARD FLOREY: CORONARY ARTERY DISEASE



Intima of a coronary artery of a pig. The picture shows the substantial cellular nature of the endothelium. Small so-called pinocytotic vesicles and mitochondria are seen in the cytoplasm. In the subendothelial space are collagen, elastic (EI), and other fine fibres (FF). The internal elastic lamina is clearly defined, and deep to this is the media. At A are attachments of smooth muscle cells of the media to the internal elastic lamina. The artery was fixed in a contracted state. It is probable that in life the endothelium would be flatter and nearer to the internal elastic lamina. (Electronmicrograph.  $\times 16,500$ .)