Medical Grand Rounds
Parkland Memorial Hospital
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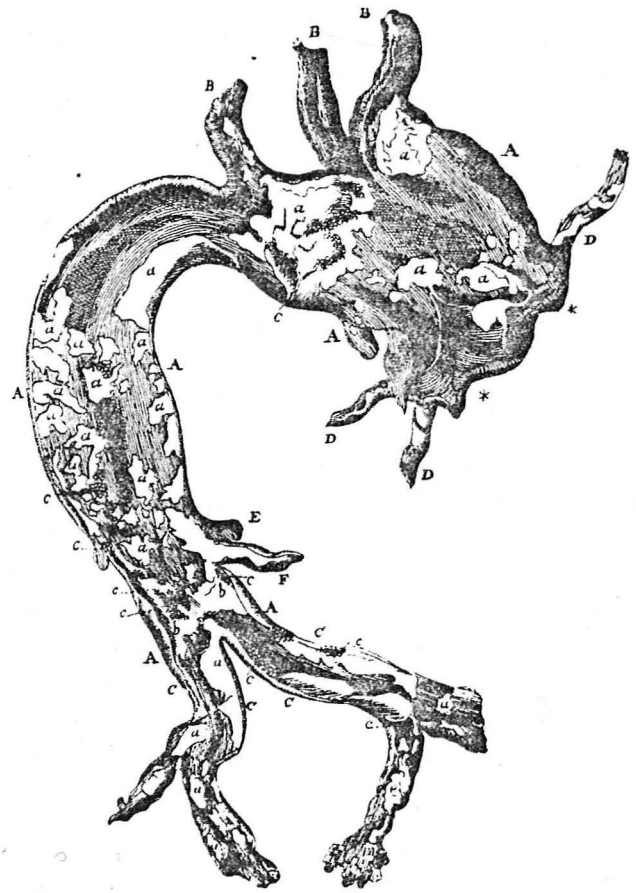
Michael S. Brown, M.D.

PROMISING NEW APPROACHES TO THE PREVENTION OF ATHEROSCLEROSIS



The aorta of Johann Jakob Wepfer (deceased 1695).

There is little mystery in Atherosclerosis. Compare it, for example, with the other great killer of industrialized man - cancer. A full understanding of cancer will require the discovery of new principles of Biology - how cell division is controlled and how genes are turned on and off. No such conceptual breakthrough is necessary to understand atherosclerosis. Its mechanism has been obvious to pathologists for more than a century.

The cover of this protocol shows a drawing of the aorta of the pathologist Johann Jakob Wepfer, who died in 1695 at the age of 75. The drawing of his aorta was included in the posthumous edition of his own pathology text. The extensive atherosclerosis is obvious (1). However, the clinical significance of the disease was then unknown.

By the mid-19th century, pathologists had an excellent conception of the pathogenesis of atherosclerosis that has changed little to this day. Figure 1 shows a drawing of an atheroma prepared by Virchow in 1858. All of the features of the disease were well described, and Virchow postulated that the lipid deposits were derived from the blood by insudation through the endothelium (2). Although this descriptive analysis of atherosclerosis has been available for 100 years we have only recently acquired sufficient insight into the underlying causes of this process to consider chemical ways of preventing the disease.

In today's Grand Rounds I will review two new approaches to the prevention of atherosclerosis.

Figure 1

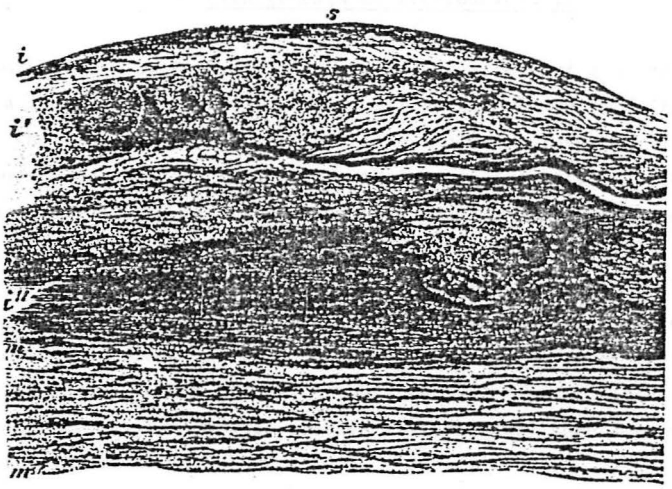
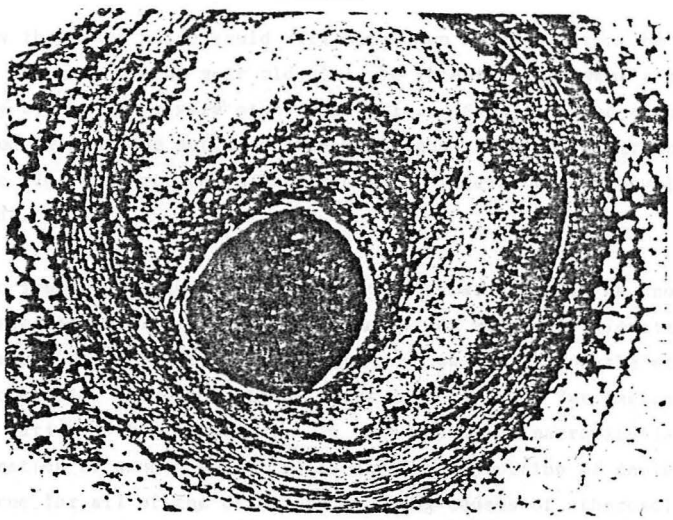


Figure 2



Pathogenesis of Atherosclerosis

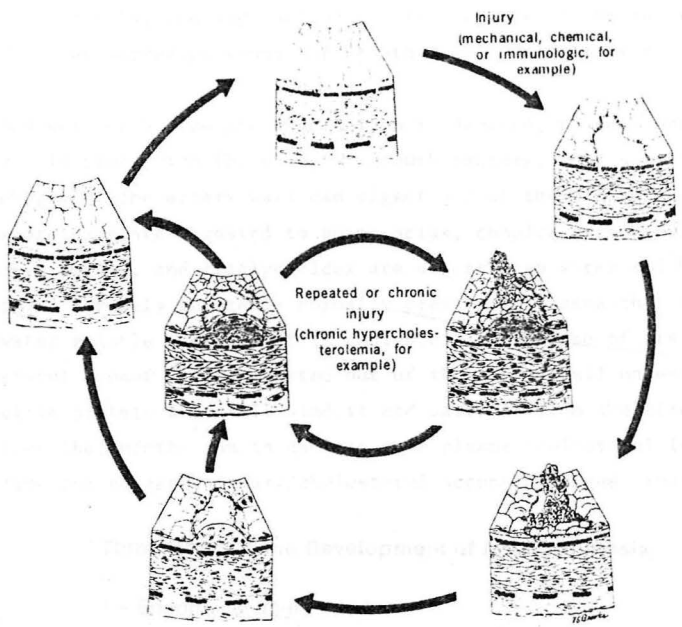
Figure 2 is a cross section of a coronary artery of a 40 year old man who died of a myocardial infarction. The wall of the artery is diffusely thickened owing to the proliferation of cells in the innermost layer, or intima. These are smooth muscle cells that migrate from their normal location in the muscular layer or media of the artery into the inner layer during the atherosclerotic process. Beneath the layer of proliferating smooth muscle cells there is a core which appears red in this photograph. This is a massive deposit of cholesterol that gives the atheroma its name. If there were only smooth muscle cell proliferation without cholesterol deposition, one would have the innocuous type of intimal thickening that occurs normally with ageing in the arteries. When cholesterol deposition takes place the lesions grow to massive size and only then does vascular obstruction occur.

The man from whom this coronary artery was obtained had a plasma cholesterol level of 220 mg/dl - clearly within the "normal" range for 40 year old men in the United States. He did not smoke, have hypertension, or diabetes. He didn't even have a type A personality. Yet over the years his plasma cholesterol deposited progressively in his coronary artery until it narrowed the lumen sufficiently to cause the formation of the thrombus that took his life.

Now this same slide could well have been obtained from the heart of another patient of ours, a nine year old girl who recently underwent a triple-vessel coronary bypass for severe atherosclerosis. She developed coronary disease in childhood because she suffered from Homozygous Familial Hypercholesterolemia. From the time of birth her plasma cholesterol level was massively elevated to 900 mg/dl.

The important point is this: In the 9 year old girl we know the etiology of the atherosclerosis. It is clearly due to her massively elevated plasma cholesterol level. In the 40 year old man, we don't know the etiology of the atherosclerosis. His cholesterol level is "normal". Yet the coronary arteries of both patients look identical. In both cases the narrowing is caused by the proliferation of smooth muscle cells and the deposition of cholesterol. The same holds true for all of the other predisposing causes of atherosclerosis. This is the unitary hypothesis of atherosclerosis. It states that no matter what the exascerbating cause might be in any individual - be it hypertension, smoking,

Fig. 3. Pathogenesis of Atherosclerosis



diabetes, or whatever - the final pathologic lesion is always the same: a proliferation of smooth muscle cells and a deposition of plasma cholesterol leading to luminal narrowing and eventual thrombotic obstruction.

This unitary hypothesis, although it seems simple, is really a powerful concept. It says that to attack atherosclerosis we can attack certain steps in the pathogenesis that are common to all forms of the disease.

What are these pathogenetic steps? Figure 3 shows a scheme for the pathogenesis of atherosclerosis as recently published by Ross and Glomset (3). This is a modernized version of a concept first elucidated by Virchow more than one hundred years ago. The hypothesis states that the initial event in atherosclerosis is an injury to the endothelium. Circulating blood platelets, which normally do not adhere to endothelium, adhere to the underlying tissue that is exposed when the endothelium is damaged. These adherent platelets release substances that stimulate the migration and proliferation of smooth muscle cells in the media. These stimulated cells cross the internal elastic lamina and enter

the intima where they proliferate and secrete collagen, elastin and other substances that repair the damaged wall. In most cases these repair mechanisms work. Eventually, new endothelium covers the site of the injury, and the only residue is an increased number of smooth muscle cells in the intima.

However, each time the endothelium is damaged, plasma constituents enter the artery wall along with the platelet growth factors. The smooth muscle cells and macrophages of the artery wall can digest all of these substances except one. Plasma proteins are digested to amino acids, complex carbohydrates are digested to simple sugars, and triglycerides are digested to water soluble fatty acids and glycerol. The only substance normally present in plasma that cannot be digested to a water soluble constituent is cholesterol. Because of its insolubility plasma cholesterol cannot be transported out of the artery wall unless it has a carrier - a specific protein that will bind it and carry it from the site of the lesion. Each time the endothelium is damaged some plasma cholesterol is left in the lesion. With time and repeated injury cholesterol accumulates and creates an atheroma.

Three Steps in the Development of Atherosclerosis

Figure 4

1 - Endothelial Injury

2 - Platelet Aggregation

3 - Deposition of Low Density Lipoprotein - Cholesterol

Figure 4 summarizes the three pathogenetic factors in atherosclerosis. In all cases atherosclerosis must involve some contribution by each of these. Moreover, these factors potentiate each other. For example, if an individual has a high plasma LDL level, then he only needs a small amount of endothelial injury in order to produce a lot of cholesterol deposition. On the other hand, an individual with a low cholesterol level would still develop atherosclerosis if he had a great deal of endothelial injury, perhaps induced by smoking or hypertension. This multistep pathogenetic process may explain the "additivity" of risk factors that has been found in the population studies of atherosclerosis.

As far as genetic factors are concerned, we know only of genetic factors that influence the plasma cholesterol level. We do not yet know about genetic factors that cause an increased susceptability to endothelial damage, or that cause an abnormally brisk platelet response. Undoubtedly such genetic variability exists, and explains many of the cases of familial myocardial infarction without known risk factors.

In designing drugs for the prevention of atherosclerosis it may be possible to attack each of these three steps separately.

Endothelial Injury

First, let us consider endothelial injury. The major evidence for the role of endothelial injury in atherosclerosis derives from the fact that atheromas are always localized. Now matter how high the blood cholesterol and what the other predisposing factors, atherosclerosis never involves the entire vascular tree, but only selected areas. The areas most frequently involved are those at the bifurcations of the arteries - that is, at points where the flow in a vessel becomes turbulent. Studies by Donald Fry and his associates at the NIH (4) have shown that these areas of turbulence are areas where the arterial endothelium is damaged by the flow of blood. This is where atherosclerosis begins.

But endothelial injury need not only be mechanical. Richard Minnick and his associates at New York Medical College have injected rabbits with foreign serum proteins (5). The animals develop circulating antigen-antibody complexes that cause the endothelium of the large arteries to slough. This sloughing leads to the aggregation of platelets. If the animal is also hypercholesterolemic, atherosclerosis will occur.

More recently, Clarkson and his associates have reported that monkeys that have undergone vasectomy develop atherosclerosis (6). The atherosclerosis is correlated with the appearance in the serum of antibodies directed against sperm. Such antibodies form routinely following vasectomy in any species. This raises the chilling possibility that men who have had vasectomies may be at increased risk for atherosclerosis, presumably because of the circulation of immune complexes in the serum. This report needs to be substantiated before any public health recommendations are made.

Three Ways to Reduce Endothelial Injury

Figure 5

1. Decrease Blood Pressure
2. Stop Smoking
3. ? Prevent immune complex circulation

Another source of endothelial injury is chemical. Ross and Harker at Seattle have infused baboons chronically with the amino acid homocystine (7). The animals develop endothelial injury. If the animals are hypercholesterolemic, atherosclerosis occurs at the site of the injured endothelium. The importance of these experiments lies in the clinical observation that patients with the genetic disease homocystinuria who have chronically high levels of homocystine in the circulation develop fulminant atherosclerosis presumably because of chemical damage to the endothelium.

How then can we reduce endothelial injury? Figure 5 shows three steps that are immediately obvious. (1) Reduce blood pressure; (2) Stop smoking (nicotine damages endothelium); and (3) Decrease the amount of immune complexes circulating. With regard to the latter point, various writers have speculated that hyperimmunization of children with vaccines might actually contribute to atherosclerosis (5). I hasten to state that there is no evidence in favor of this suggestion.

Let us now turn to the second factor in the pathogenesis of atherosclerosis - platelet aggregation. As yet, there is no direct and conclusive evidence that platelets play a role in the formation of atherosclerotic plaques. However, over the past few years a great deal of circumstantial evidence has been accumulating that is suggestive in this regard. The first evidence was the observation by Ross and co-workers that platelets contain a growth factor that stimulates the growth of aortic smooth muscle cells in tissue culture (3). This growth factor is a protein that is released from platelets when they aggregate. This observation may offer an explanation for the smooth muscle cell proliferation that occurs in atheromas. The hypothesis is that the endothelium is damaged, platelets adhere, and they release the growth factor. This causes the smooth muscle cells to migrate from the media into the intima, to proliferate, and to initiate atherosclerosis.

Recently an experiment was described that provides further indirect evidence in support of the platelet hypothesis. There is a strain of pigs with a genetic disease that is analagous to von Willebrand's disease. These pigs' platelets do not function properly. The animals have prolonged bleeding times and frequently bleed to death. When normal pigs are put on a high cholesterol diet, they develop hypercholesterolemia and atherosclerosis. However, when the von Willebrand's pigs are put on a high cholesterol diet they develop equal hypercholesterolemia, but they fail to develop atherosclerosis (8).

Figure 6 shows the aorta of a normal pig that was fed a high cholesterol diet (8). The intima is severely thickened because of the proliferation of smooth muscle cells. In addition, there are tremendous lipid deposits within and surrounding these smooth muscle cells.

Figure 7 shows a fat stain of the aorta of a von Willebrand's pig who was put on the same diet. There are tremendous lipid deposits throughout the aorta. However, when the aorta is sectioned histologically (Fig. 8), one sees that the lipid has deposited in the intima, but the intima has not responded with any cellular proliferation. In the absence of normal platelet function one gets an aorta that is loaded with cholesterol but which has no cellular proliferation.

This intriguing study raises many questions. First, it must be repeated to determine whether it is correct. The von Willebrand's pigs are not healthy in many ways. For example, they weighed much less than the control animals in these studies and this general unhealthiness may retard their smooth muscle proliferation. Moreover, scoring of these atherosclerotic lesions is necessarily subjective and this study must be repeated by another group before it can be accepted. Finally, much longer term feeding studies have to be done, if the animals can be kept alive. The problem is that the von Willebrand's pigs tend to die from bleeding.

Nevertheless, if all of these questions can be answered satisfactorily, the von Willebrand's study will have provided evidence that platelets are involved in the smooth muscle cell proliferation of atherosclerosis. The next question is: What about all this lipid that deposits in the absence of smooth muscle cell proliferation? Is this good or bad? Is the smooth muscle cell proliferation a healing event? What will happen to these lipid-laden aortas with time if they do not have smooth muscle proliferation? We need to know the answers.

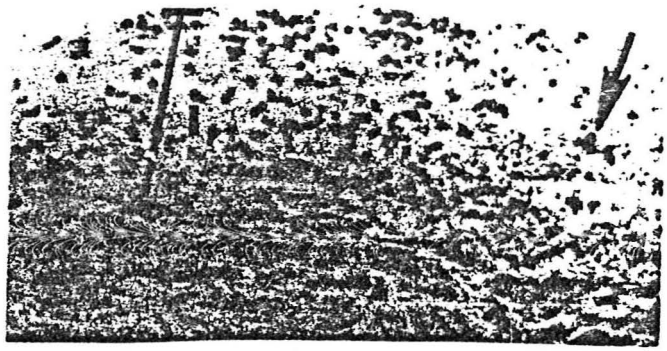
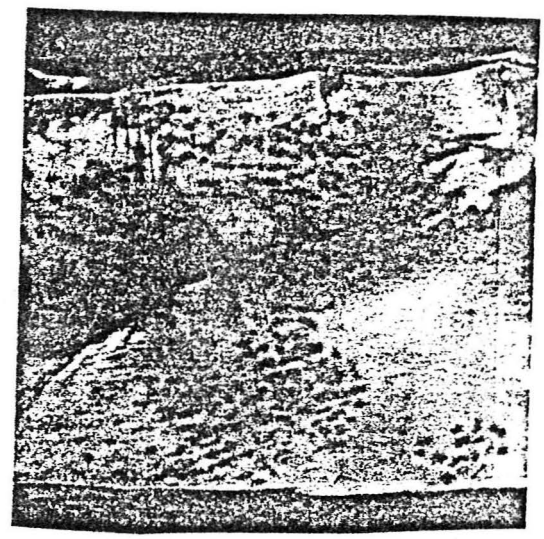


Fig. 6. Aorta of a normal pig fed cholesterol.

Fig. 7. Aorta of von Willebrand's pig fed cholesterol (Sudan stain).



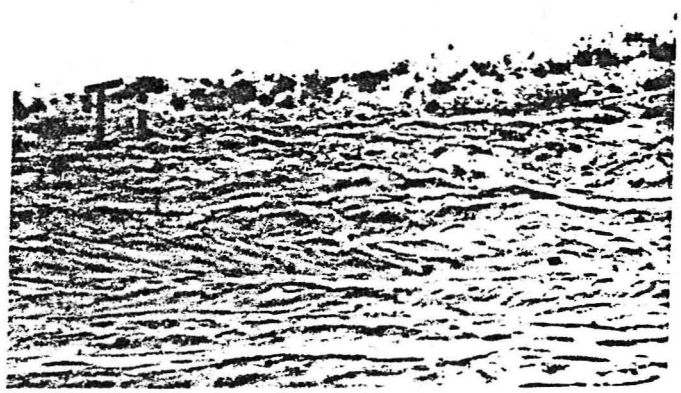


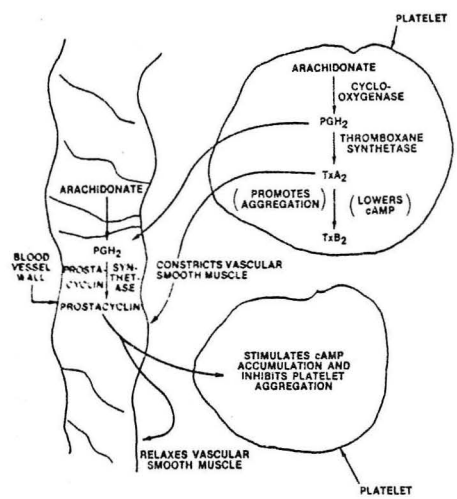
Fig. 8. Aorta of von Willebrand's pig fed cholesterol.

While the experimental studies showing a role of platelets in atheroslerosis are still in their infancy, the field of platelet metabolism has advanced so rapidly that it has already begun to explain some of these observed events at a biochemical level. These new biochemical studies have suggested ways in which platelets can be inactivated pharmacologically so that we can reproduce in man a situation resembling the von Willebrand's pig. The new breakthroughs have come in the area of prostaglandin research.

Prostaglandins And Platelets

Figure 9 summarizes the recent conception of prostaglandin metabolism as it relates to platelet interaction with vascular endothelium. Several years ago Samuelsson discovered that platelets produce a specific type of prostaglandin derivative called thromboxane (9). Thromboxane, like other prostaglandin - type compounds is derived from a fatty acid (arachidonic acid). When platelets aggregate they secrete thromboxane. The thromboxane diffuses locally and stimulates other platelets to join the aggregation and so causes the size of the platelet plug to increase. Thromboxane is essential for platelet thrombose formation. If one could block thromboxane production one could presumably prevent platelet aggregates from forming, and one might prevent atherosclerosis.

Figure 9



This all seemed simple until 1976 when Vane discovered another prostaglandin-type compound that opposes the action of thromboxane (10). This new compound is called prostacyclin.

Prostacyclin is not produced by platelets. Rather, it is produced by the endothelium of blood vessels. Prostacyclin inhibits platelet aggregation. Thromboxane and prostacyclin thus constitute an opposing system. When platelets adhere to damaged endothelium they produce thromboxane and that causes the platelet plug to increase in size. As the growing platelet plug approaches the normal endothelium at the edge of the lesion, the platelets become exposed to prostacyclin which is produced by the normal endothelial cells. This prevents further platelet aggregation and limits the size of the platelet thrombus. Vane has further speculated that continuous prostacyclin production is the factor that prevents platelets from adhering to normal endothelium all the time.

This push-pull opposition of thromboxane and prostacyclin creates a problem for pharmacologists. To prevent platelet aggregation we must prevent the synthesis of thromboxane. On the other hand, we cannot afford to prevent the synthesis of prostacyclin. Yet both prostacyclin and thromboxane arise from arachidonic acid and both use the cyclo-oxygenase enzyme. Thus, any drug which inhibits the platelet cyclo-oxygenase and thereby prevents thromboxane formation also has the potential of inhibiting prostacyclin formation and increasing platelet aggregation.

It should also be noted that prostacyclin acts by raising the Cyclic AMP level of the platelet. Other agents that raise the Cyclic AMP level of the platelet also inhibit aggregation.

Recall that platelets are believed to act at two stages in clinical atherosclerosis. First, they <u>initiate</u> the formation of atheroma by stimulating smooth muscle cell division. And second, platelets act <u>terminally</u> by forming a thrombus that occludes the vessel.

Anti-platelet drugs have to be tested in both of these stages. First, we have to find out whether anti-platelet drugs prevent thrombosis in people who already have atherosclerosis. And second, we have to find out whether anti-platelet drugs prevent the formation of the atherosclerotic plaque itself.

Figure 10

Anti-Platelet Drugs

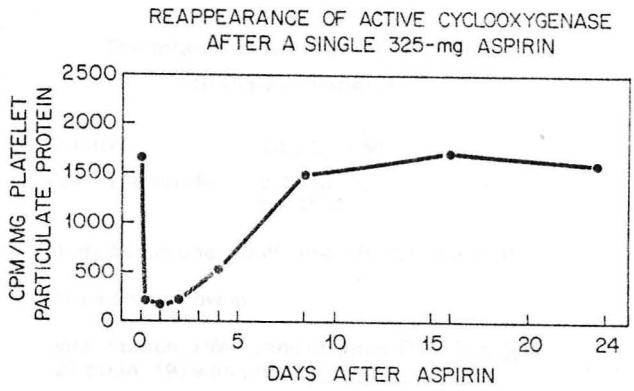
Drug	Mode of Action
1. Aspirin	Inhibits Cyclo-oxygenase
	Low Doses: Inhibits thromboxane synthesis in platelets.
	High Doses: Inhibits prostacyclin synthesis in endothelium.
2. Dipyridamole (Persantine)	Inhibits phosphodiesterase (increases platelet cyclic AMP)
3. Sulfinpyrazone (Anturane)	? Inhibits prostaglandin synthesis

Figure 10 lists three of the anti-platelet drugs that have received the most clinical attention in man - aspirin, dipyridamole and sulfinpyrazone. The most well-studied of these is aspirin. In 1967 Weiss reported that platelets from patients who consumed aspirin showed diminished aggregation (11). Vane subsequently found that aspirin acted by inhibiting the platelet cyclo-oxygenase enzyme, thereby preventing the formation of prostaglandins and thromboxane (12). Majerus then showed that aspirin permanently inactivated the cyclo-oxygenase by acetylation (13). The acetyl group was transferred from acetylsalicyclic acid to the cyclo-oxygenase enzyme. Aspirin inactivated the cyclo-oxygenase at extremely low concentrations in vitro.

Clinically, the importance of Majerus' discovery was that the acetylation of the enzyme is irreversible. Mature platelets do not synthesize any new cyclo-oxygenase and so they cannot replace the cyclo-oxygenase that is inactivated. One dose of aspirin will therefore cause a prolonged inactivation of the cyclo-oxygenase of megakaryocytes and circulating platelets. New enzyme will not appear until new platelets have been synthesized - that is, after about 9 days.

Figure 11 shows the response to a single aspirin tablet in a group of normal volunteers (14). Platelet cyclo-oxygenase was immediately inactivated and the enzyme remained inactivated until new platelets appeared at seven to nine days.

Figure 11



Data of Majerus and Co.

Majerus' findings also suggest a way to inhibit thromboxane production by platelets without affecting prostacyclin production by the endothelium. Much higher levels of aspirin are required to inhibit the cyclo-oxygenase of endothelial cells as compared with the cyclo-oxygenase of platelets. Moreover, since endothelial cells continually synthesize new protein they replace their acetylated cyclo-oxygenase rapidly and do not show the prolonged depression seen with aspirin.

The clinical lesson of these studies is clear: In using aspirin as an anti-platelet agent one should give extremely low doses which are just sufficient to inactivate the cyclo-oxygenase of platelets and prevent thromboxane production but not high enough to inactivate the cyclo-oxygenase of endothelium and prevent prostacyclin production.

Clinical Studies of Anti-Platelet Agents

Will such low dosages of aspirin be clinically effective against platelet action in man? Majerus has just concluded a study that indicates that it will. Figure 12 shows an experiment with patients on chronic hemodialysis who had arteriovenous shunts (15). The patients were randomized into two groups. One received aspirin at the low dose of one-half tablet daily and the other group received a placebo. The study was begun one month after the shunts were placed and the patients were followed for an additional five months. The code has just been broken, and the results will appear shortly in the New England Journal of Medicine. In the control group 70% of the A-V shunts developed thrombosis over the five month period. In the group receiving aspirin, only 33% of the shunts thrombosed. This reduction was highly significant statistically. Thus, aspirin in extremely low doses can help prevent platelet aggregation and thrombosis clinically in man.

Figure 12

Thrombosis of Arteriovenous Shunts
In Dialysis Patients

Control	18/25 (72%)
ASA (½-tablet/day)	6/19 (33%)
	$p < 0.01$

Study begun one month after shunt placement.

Five-month followup.

Data of Burch, J.W. . . . and Majerus, P.W., Clin. Res. 27:509A, 1979 (in press, N. Engl. J. Med.)

Will aspirin prevent arterial thromboses in patients with atherosclerosis? We don't yet know for sure. Such studies are difficult because of the variable nature of the atherosclerotic process. However, at least two prospective trials have shown some reduction in thrombotic events in men who take aspirin - one study on potential MI patients and the other on candidates for cerebral vascular accidents.

Figure 13

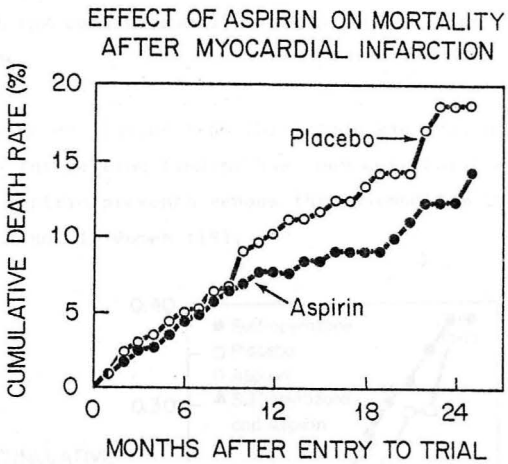


Figure 13 shows the results of the British MI study reported in 1974 (16). 1200 men were placed on one aspirin tablet daily or placebo following a myocardial infarction and their subsequent mortality was followed. At every time interval up to 24 months mortality was lower in the aspirin group than in the placebo group.

However, surprisingly, these results did not reach the level of statistical significance. One of the interesting findings was that aspirin was most effective when it was started within six weeks of discharge from the hospital following a myocardial infarction. Although this study did not show statistical significance by the methods employed, the data were highly suggestive. Moreover, in the same issue of the British Medical Journal, a Boston group reported in a retrospective study that the incidence of regular aspirin use was lower in MI patients than in other hospitalized patients (17). Together, these studies raised the possibility that aspirin may be beneficial in the secondary prevention of thrombosis among patients who have already had a myocardial infarction.

Last summer another study was reported (18). This one was from Canada and involved stroke patients (Fig. 14). Patients with transient ischemic attacks were treated with two anti-platelet drugs alone or in combination. The two drugs were aspirin and sulfinpyrazone. They were then followed for three years to determine whether these drugs would prevent the occurrence of a stroke. Figure 14 shows that aspirin significantly lowered the cumulative probability of stroke or death among the men by approximately 50% up to 42 months. Sulfinpyrazone by itself had no effect. But the combination of aspirin plus sulfinpyrazone was more effective than aspirin alone.

A striking conclusion from this study was that neither of the drugs benefited women. This interesting finding has been corroborated by another study which showed that aspirin prevents venous thromboembolism in men who have had a hip fracture, but not in women (19).

Figure 14

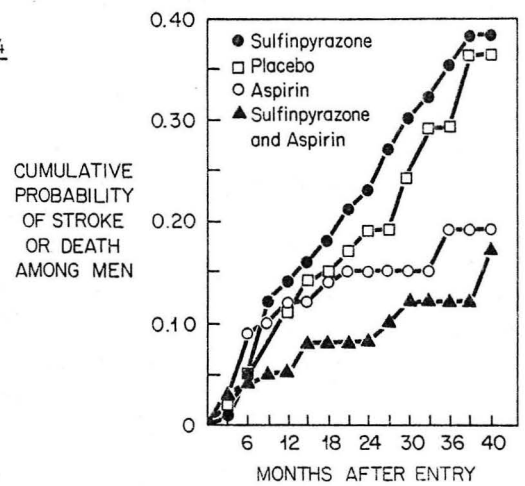
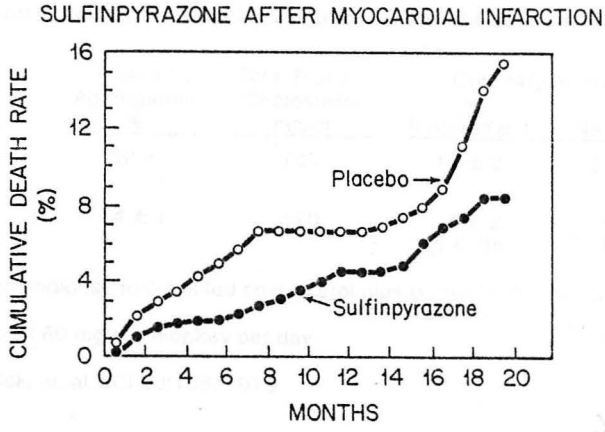


Figure 15



Whereas the Canadian study of sulfinpyrazone in stroke patients was negative, another study showed a highly significant effect of sulfinpyrazone on the prevention of cardiac death after myocardial infarction (Fig. 15). In this group, the death rate was reduced by 57% in those taking sulfinpyrazone as opposed to the control group (20). Sulfinpyrazone had its greatest effect by reducing the occurrence of sudden death. The number of patients followed for the longer periods was small and it remains to be seen whether this lower mortality rate will be continued for longer periods. As far as I am aware, a follow-up has not been reported.

These studies of anti-platelet agents are suggestive but not terribly conclusive. They all have been "secondary" prevention trials. That is, the investigators start with people who already have symptoms of ischemia or infarction. Such individuals may have lesions that are too far advanced for significant benefit. The real question is whether aspirin will have an effect on individuals who have atherosclerosis but have not yet developed symptoms of vascular disease. This requires a so-called "primary" prevention trial, which is much more difficult than a secondary trial. None have yet been reported for any anti-platelet agent.

Figure 16

Effect of Aspirin on Platelet Aggregation, Plasma Cholesterol And Coronary Atherosclerosis in Cholesterol-Fed Monkeys

Group	Platelet Aggregation %	Total Plasma Cholesterol mg/dl	Coronary Arteries % Involvement	Coronary Arteries % Narrowing
Control (n = 6)	52 ±	700	14 ± 2	31 ± 5
Aspirin (n = 5)	4 ± 1	570	5 ± 2 p < .05	15 ± 6 N.S.

Young Cynomolgus monkeys fed cholesterol plus butter for 6 months.

Aspirin dose: 80 mg per monkey per day.

Data of Pick, et. al. JCI 63:158, 1979.

The question then arises: Is there any evidence that antiplatelet drugs can prevent the occurrence of atherosclerosis itself? Figure 16 is derived from a study reported this January by Pick and associates (21). These workers fed a high cholesterol diet to monkeys. One group was treated with aspirin and the other group received a placebo. At the end of six months the monkeys were killed and the extent of cholesterol-induced atherosclerosis was determined. Both groups had a comparable rise in the plasma cholesterol level to the range of 570-700 mg/dl. In the group taking aspirin platelet aggregation was inhibited. The aspirin-treated group had a much lower involvement of the coronary artery with atherosclerotic plaques. Moreover, the per cent luminal narrowing for each plaque was reduced in the aspirin group as compared with the controls. These striking findings must be corroborated before they can be accepted. Again, they involve somewhat subjective measurements of atherosclerosis in animals. Nevertheless, when coupled with the data in the von Willebrand's pigs, they at least raise the possibility that an interference with platelet function will retard the development of atherosclerosis in the presence of hypercholesterolemia.

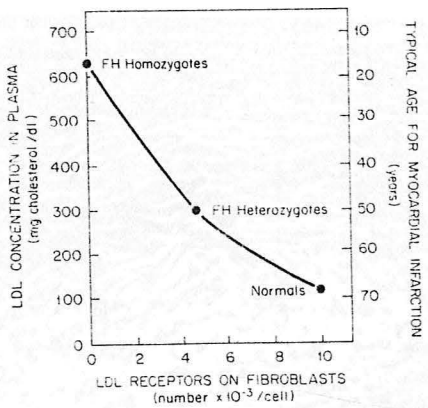
Plasma LDL-Cholesterol

Let us now turn to the third aspect of the pathogenesis of atherosclerosis and the third point of therapeutic attack. This is the plasma LDL-cholesterol level. What is the evidence that plasma LDL-cholesterol has anything to do with the pathogenesis of atherosclerosis? This evidence is listed below:

Evidence for the Role of Plasma Cholesterol in Atherosclerosis

1. Full-blown atherosclerosis can be produced in every species of animal simply by feeding a diet that raises the plasma cholesterol level.
2. Whereas a proliferative thickening of the artery wall can be produced in animals by trauma to the wall itself (such as by producing high blood pressure or denuding the endothelial lining by physical means), the lesion resembles a benign scar unless the animal's blood cholesterol level is elevated, in which case the lipid is deposited in the lesion and full-blown, life-threatening atherosclerosis ensues.
3. In human populations atherosclerosis does not frequently develop, even in the face of predisposing factors such as diabetes mellitus, smoking, and hypertension, unless the mean plasma cholesterol of the population is greater than 160 mg/dl.
4. Within any single population the probability of an individual developing the most common complication of atherosclerosis, myocardial infarction, increases in proportion to his plasma cholesterol level.
5. Single gene-determined disorders that elevate the plasma or tissue cholesterol level, such as familial hypercholesterolemia or cholesteryl ester storage disease, produce fulminant atherosclerosis in childhood without the need for any other factors such as hypertension, smoking, or diabetes mellitus.

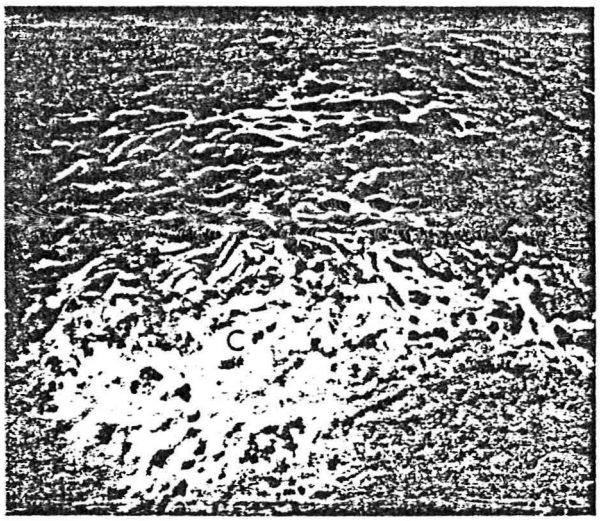
Figure 17



The relation between plasma LDL-cholesterol levels and myocardial infarction is illustrated graphically in Figure 17 which compares the age at myocardial infarction with plasma LDL-cholesterol level and the number of LDL receptors as determined on cultured fibroblast among patients with Familial Hypercholesterolemia (22). Normal subjects whose fibroblasts can develop about 10,000 LDL receptors per cell have plasma LDL-cholesterol levels of about 120 mg/dl and tend to suffer myocardial infarctions in their 60's. Patients with the heterozygous form of Familial Hypercholesterolemia, whose fibroblasts have about a half-normal number of LDL receptors have a two-fold elevation of plasma LDL cholesterol and develop M. I.'s at age 50. And finally, patients with homozygous Familial Hypercholesterolemia whose cells fail to produce LDL receptors have a massive elevation in plasma LDL levels and develop myocardial infarctions in childhood.

In addition to the extensive correlative data, of which Figure 17 is but one example, direct studies have shown that plasma LDL is the source of the cholesterol in atheromatous plaques. Figure 18 is taken from the work of Hoff (23). It shows a section of an atheroma from a man with a normal plasma LDL level. The atheroma was stained with a fluorescent antibody to apoprotein B, the protein that is present in LDL. There is marked fluorescence in the core of the atheromatous plaque, indicating that this plaque is filled with plasma LDL. Similar results have been obtained in every atheromatous plaque so far examined. Moreover, it has been possible to extract the LDL from these atheromatous plaques and to estimate the amount of lipoprotein that is present. Hoff (24) and Smith (25) have both shown that atheromatous plaques contain LDL in amounts that exceed by many-fold the concentration of LDL in plasma.

Figure 18. Immunofluorescent staining of LDL in a human atheroma.

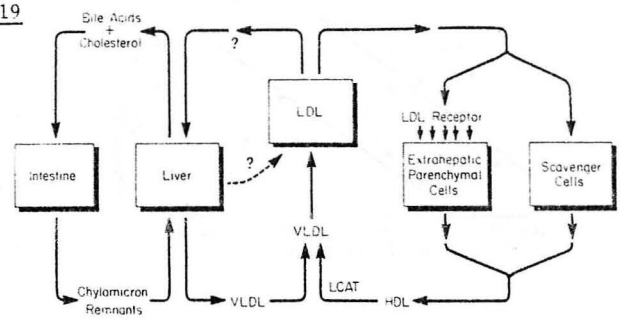


What is the "Normal" Level of Plasma Cholesterol?

In the case of hypercholesterolemia patients, it is clear why plasma LDL deposits in the artery wall - the level of LDL in the blood is too high. However, the question that we address this morning does not relate to these patients. We now ask why "normal" people with normal LDL levels have such massive deposition of LDL-cholesterol in their arteries and atherosclerosis.

Dr. Goldstein and I have suggested that this occurs because the "normal" level of LDL in plasma is unphysiologically high and in a range that prediposes to atherosclerosis (26). How do we know what the physiologic concentration of plasma LDL should be? A suggestive answer has emerged from studies of the receptor for plasma LDL. Figure 19 shows the metabolic role of plasma LDL as it has emerged from these studies. Recall that LDL is derived from the triglyceride-transporting lipoprotein VLDL which is secreted by the liver. The function of LDL is to carry cholesterol from the liver and intestine to the rest of the body cells. In delivering this cholesterol to body cells, the LDL first binds to a high affinity receptor on the cell surface.

Figure 19



Dr. Goldstein and I and our associates have studied cell surface LDL receptors from a number of tissues of man and animals. We have found that the LDL receptors from several species all have about the same affinity for LDL. Knowledge of this affinity allows us to estimate the normal level of LDL that this receptor is adapted to seeing. The LDL receptor binds LDL most efficiently at an LDL concentration of 2.5 mg/dl. This presumably is the LDL concentration that should be maintained in the interstitial fluid surrounding the receptor. The LDL receptor studies tell us the appropriate concentration of LDL in interstitial fluid, not in plasma. How can we translate this interstitial fluid level to the appropriate plasma concentration of LDL?

To make this translation, we need to know the relation between the plasma concentration of LDL and the interstitial fluid concentration. We have made these measurements and the results are shown in Figure 20 (27). To do these experiments, we obtained lymph from the dorsum of the human foot. The composition of this lymph approximates that of interstitial fluid. To measure how much LDL was present in these minute samples of lymph we tested the ability of the lymph to compete for the binding of ^{125}I-labeled LDL to the LDL receptor in fibroblast monolayer cultures.

Figure 20. Comparison of the ability of whole human serum and whole human lymph to compete with ^{125}I-LDL for binding to LDL receptor of human fibroblasts.

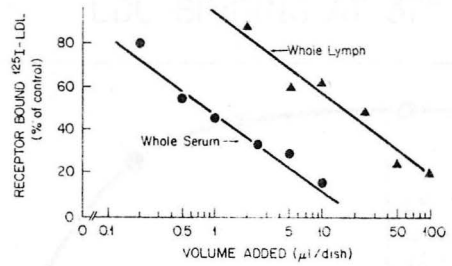


Figure 20 shows the results of such an experiment. The line on the left shows the percent displacement of ^{125}I-LDL binding when we added whole serum to the assay. About 1 microliter of whole serum was required to reduce the binding by 50%. The line on the right shows the amount of lymph from the same individual that was required to compete for ^{125}I-LDL binding. The amount required for 50% competition was about 10 µl. Thus, the concentration of biologically active LDL in the lymph of this subject was one-tenth the concentration in the plasma. Similar results were obtained with several other subjects. The results are illustrated schematically in Figure 21.

Figure 21

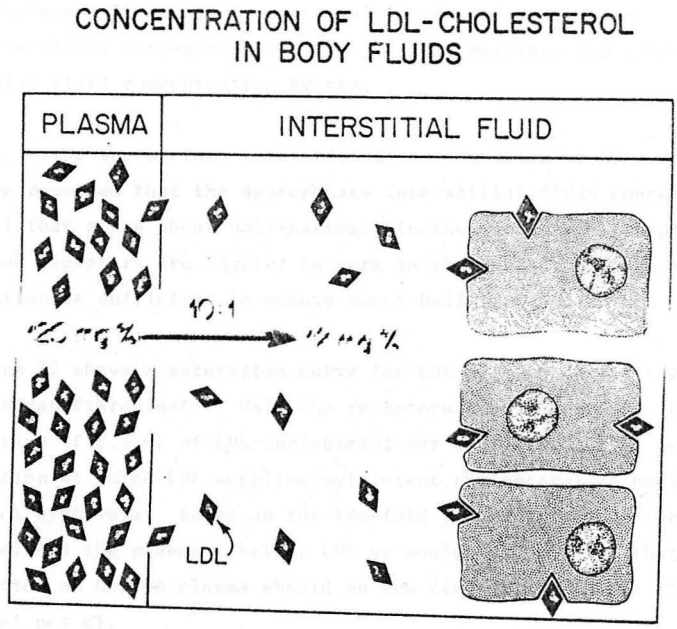
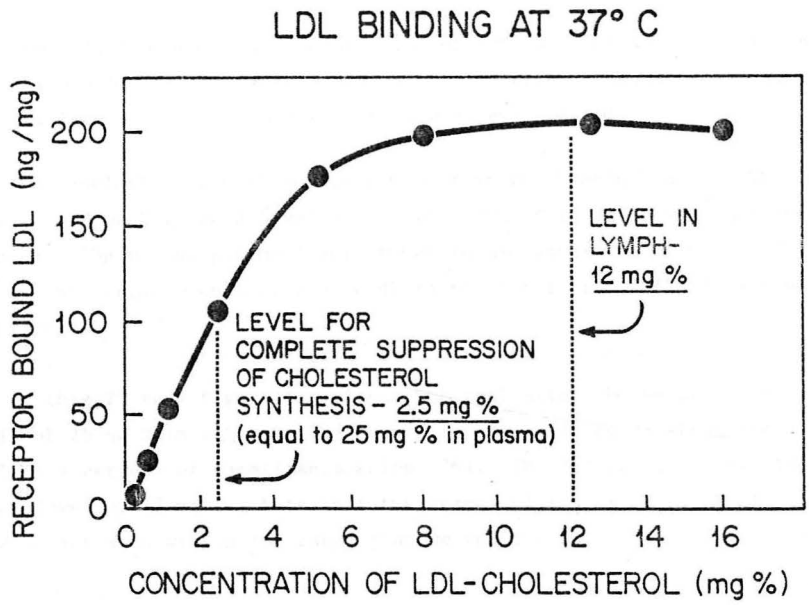


Figure 22



Because of the large size of the LDL particle it is filtered relatively poorly through the capillary endothelium and thus a ten-fold concentration gradient develops between plasma and interstitial fluid. If we want to calculate the appropriate plasma concentration of LDL we must multiply the appropriate interstitial fluid concentration by ten.

What is the appropriate interstitial concentration of LDL? Dr. Goldstein and I have reasoned that the appropriate interstitial fluid concentration would be a level that gives about half-maximal binding to the LDL receptor. In general, enzymes and receptors are adapted to work in the range where the ambient ligand concentration is sufficient to occupy about half the sites.

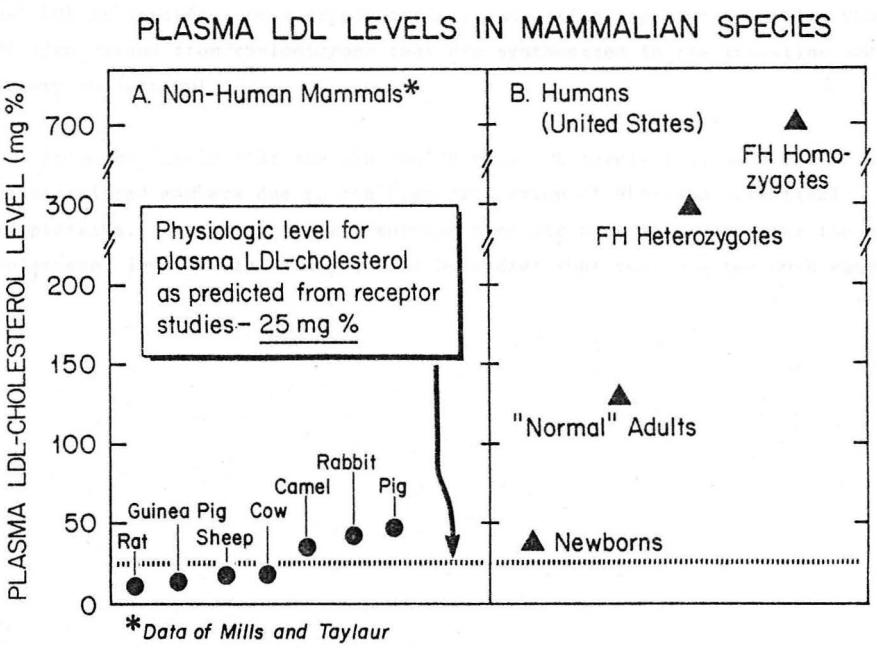
Figure 22 shows a saturation curve for LDL binding to the receptors on cultured human fibroblasts. Half the receptors are occupied at an LDL concentration of 2.5 mg of LDL-cholesterol per deciliter. This is also the concentration at which LDL supplies sufficient cholesterol to maximally suppress cholesterol synthesis. Based on the ten-fold gradient between the interstitial fluid level and the plasma level of LDL we would then predict that the appropriate concentration of LDL in plasma should be ten times 2.5 or about 25 mg of LDL cholesterol per dl.

However, the measured concentration of LDL in lymph was equal to about 12 mg %, which corresponds to a plasma LDL cholesterol level of 120 mg %. This is about 5-fold higher than the appropriate level of LDL.

The conclusion from these studies is that the "normal" plasma LDL-cholesterol level of 120 mg % gives a level of LDL in lymph of 12 mg %, which is about 5-fold too high. The normal plasma level of LDL in man should be 25 mg %. This would give an appropriate level of 2.5 mg/dl in the interstitial fluid that bathes the receptors.

Is this 25 mg % figure for plasma LDL realistic? Do we ever see plasma LDL levels of 25 mg % in nature? The answer is yes. Figure 23 shows the plasma LDL level in a variety of mammalian species (26). The dashed line shows the predicted normal level of 25 mg %. Note that the normal plasma level of LDL in all of these animal species is within the range that we would predict to be physiologic for man.

Figure 23

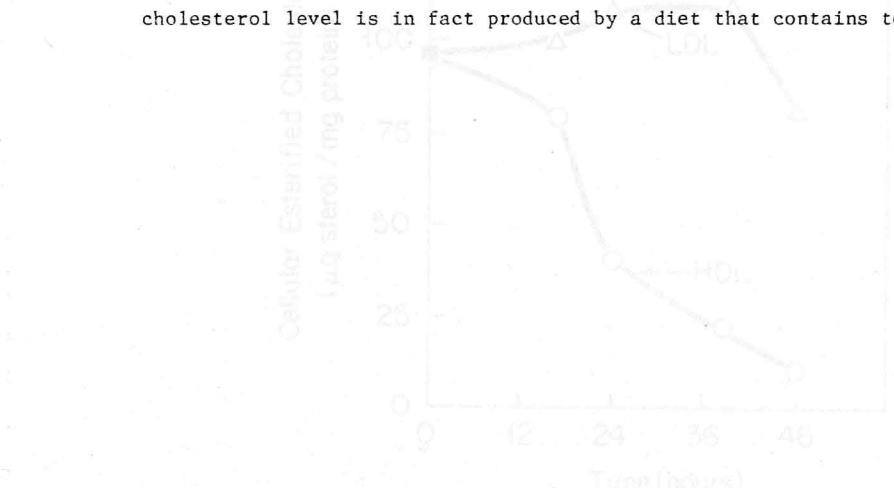


The right hand side of this figure shows the situation among men in the United States. At birth the plasma level of LDL cholesterol is about 30 mg %, right in the physiologic range predicted from the LDL receptor studies. But after birth something happens to the LDL level to lift it above this physiologic range. In normal Americans the level goes up to about 120 mg per dl, or about 5-fold above the level that we would consider physiologic. Of course, in familial hypercholesterolemia heterozygotes and homozygotes the level is even more markedly elevated.

This elevation to 120 mg per dl that we consider pathologic does not occur in all men. It only occurs in a small subset of men that populates Western Europe, North America and a few other industrialized areas of the world (28). It does not occur among the masses of people in less developed nations who depend on fishing or farming rather than food stores for their daily sustenance.

To understand why the plasma LDL level is so high among normal industrialized men, let us return to the pathway for cholesterol transport (Figure 19). Note that most LDL is derived from a triglyceride transporting lipoprotein called VLDL. Some LDL also arises from chylomicrons that are synthesized in the intestine and carry dietary cholesterol.

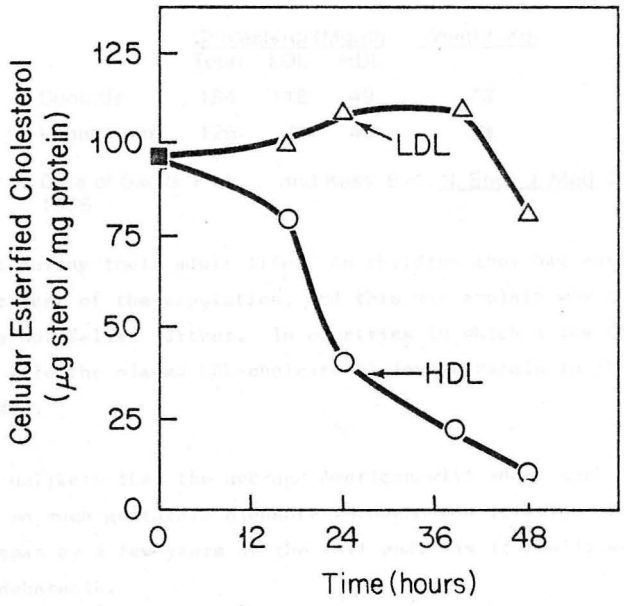
It seems likely that the abnormally high LDL levels that we normally see in industrialized man are due to the high production of VLDL and intestinal lipoproteins. Extensive dietary surveys have strongly suggested that the high cholesterol level is in fact produced by a diet that contains too much saturated



fat, too much cholesterol, and too many calories (28). This drives LDL production above the physiologic range. When the tissue receptors become saturated the LDL level rises still higher. Much of the excess LDL is metabolized in the reticuloendothelial system and other scavenger cells. Some of this excess LDL also deposits in arteries and produces atherosclerosis.

Several weeks ago in his Grand Rounds, Dr. Goldstein showed you how LDL that was altered by a certain chemical reaction, acetylation, was able to deposit cholesterol in macrophages and other scavenger cells. We have also studied the other side of the coin. It turns out that high density lipoprotein or HDL can remove that cholesterol that has been deposited in macrophages by LDL. Figure 24 shows an experiment in which macrophages were first incubated with acetyl-LDL so that they accumulated large amounts of cholesterol and then were incubated with increasing amounts of HDL. The HDL was able to remove all the cholesterol that had accumulated. In contrast LDL was not able to remove this cholesterol. The ability of HDL to remove cholesterol from scavenger cells may explain in part the epidemiologic finding that high HDL levels are associated with a protection from atherosclerosis.

Figure 24. High Density Lipoprotein, but not Low Density Lipoprotein, removes cholesterol from cholesterol-laden macrophages in vitro.



Lowering the Plasma Cholesterol Level

If the epidemiologic and biochemical studies are correct the plasma LDL cholesterol level in American men really is much too high. How can we lower it? One answer is diet. But not the casual kind of dietary gesture that we usually tell our patients about. That is, substituting lean meat for fatty meat and margarine for butter. In order to lower the cholesterol into a really physiologic range we have to go back on the diet that we are apparently adapted to by evolution - that is, primarily fruits and vegetables with only a tiny smattering of meat.

Figure 25 shows that such a diet does work. Plasma cholesterol levels were compared among a group of vegetarians living in a commune in Massachusetts and a group of age and sex matched controls eating a normal diet. The mean cholesterol level in the controls was 184 mg/dl and in the vegetarians it was 126 mg/dl. Most important, the difference in cholesterol was due almost entirely to a reduction in the LDL cholesterol level from 118 to 73 mg/dl. This is getting close to the postulated physiologic range for man. These vegetarians had only been on such a

Plasma Lipids and Weights in Massachusetts Vegetarians vs. Age and Sex-matched Controls

Figure 25

	Cholesterol (Mg/dl)			Weight (Kg)
	Total	LDL	HDL	
Controls	184	118	49	73
Vegetarians	126	73	43	58

Data of Sacks, F.M. . . . and Kass, E.H., N. Engl. J. Med. 292:1148, 1975

strict diet during their adult life. As children they had eaten the same high fat diet as the rest of the population, and this may explain why their LDL-cholesterol levels have not fallen further. In countries in which a low fat diet is consumed throughout life the plasma LDL-cholesterol levels remain in the range of 40 to 50 mg/dl (29).

It is unlikely that the average American will adopt such a strict diet. To give up so much gustatory pleasure throughout a lifetime in order to extend one's lifespan by a few years at the tail end. Is it really worth it? Certainly debatable.

Our job as physicians and scientists is to find a way to prevent atherosclerosis while our patients are still eating an enjoyable diet. Can this be done?

Until now it has not been possible. No drug has been able to normalize the plasma cholesterol level while patients are on their usual high cholesterol, high fat diets. But recently a breakthrough has been made that may change all this. The breakthrough involves a drug called Compactin which inhibits cholesterol synthesis.

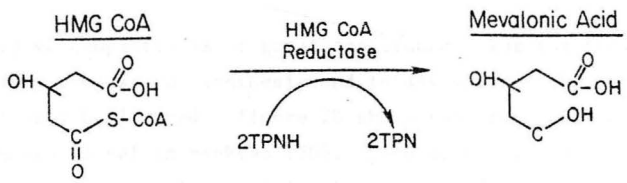
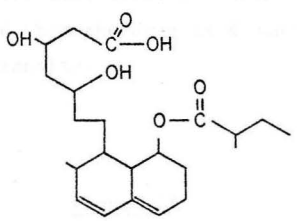


Figure 26



Compactin

Compactin, An Inhibitor of Cholesterol Synthesis

Figure 26 shows the structure of Compactin. It was discovered in 1976 by a very clever Japanese scientist, Akira Endo. Dr. Endo knew that the most important enzyme in cholesterol synthesis is HMG CoA reductase. This enzyme converts HMG CoA to mevalonate, which is in turn converted to cholesterol. Mevalonate is required by all cells in nature for synthesis of other polyisyrenoids as well as cholesterol. Hence, HMG CoA reductase is found in all cells. Dr. Endo reasoned that this enzyme was so important that somewhere some fungus must have made an inhibitor of the enzyme to kill its enemies. Endo patiently analyzed extracts from 6,000 different fungal strains and found 1 strain that made a substance that inhibited the enzyme. The structure of this inhibitor is shown in Figure 26.

Compactin has two rings and a side chain that is almost an exact copy of HMG CoA, the natural substrate for HMG CoA reductase. The affinity of the enzyme for compactin is 10,000-fold higher than its affinity for the natural substrate. Therefore, compactin is an extremely potent competitive inhibitor of the reductase. The compound is exactly analagous to penicillin and other antibiotics that are produced by fungi to inhibit critical enzymes in their enemies' cells.

Figure 27 shows that tiny doses of compactin completely inhibit cholesterol synthesis in cultured mammalian cells. Similar results have been obtained in cultured human fibroblasts and freshly isolated human lymphocytes.

The discovery of compactin is of great importance. For the first time it allows us to inhibit cholesterol synthesis and to ask whether the plasma cholesterol level will be lowered. Figure 28 shows the effect of compactin on the plasma cholesterol level in monkeys (30). When given orally in a single dose daily it reduces the plasma cholesterol level in these normal animals by 40% and this effect is promptly reversed when the drug is discontinued. Similar results have been obtained in dogs and rabbits.

Figure 27

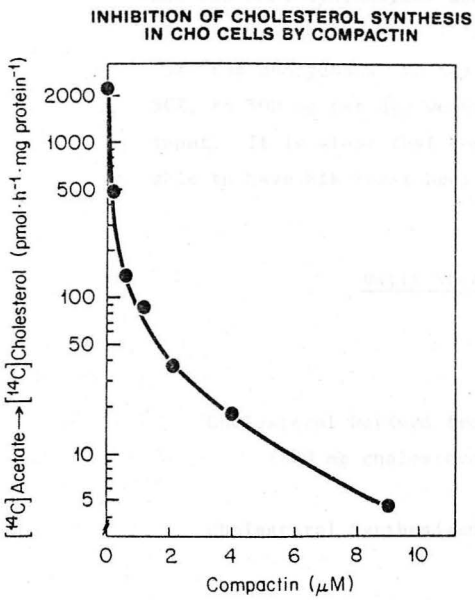
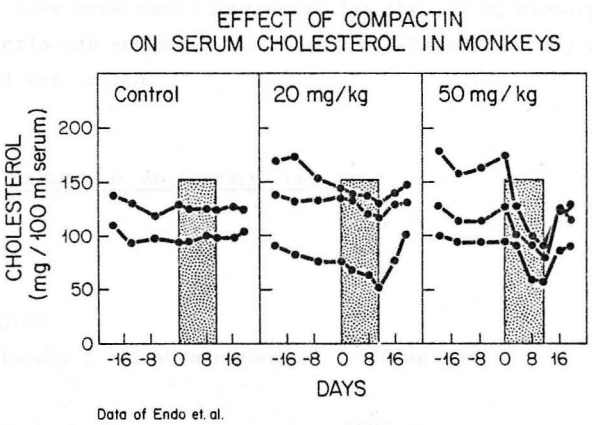


Figure 28



Compactin is now undergoing extensive clinical trials in Japan. The results are not yet published, but personal communications indicate that the drug is extremely effective in lowering cholesterol levels in normal individuals as well as in patients with a variety of hypercholesterolemic states. Moreover, extremely recent evidence shows that certain derivatives of compactin exist in nature that are 10-fold more potent than the original compactin compound. No clinical toxicity has been observed from any of these compounds in man, and the drugs have passed the Ames test for mutagenicity.

We are now faced with a paradox. On one hand we're saying that the hypercholesterolemia of Western man is caused by excess dietary cholesterol and fat; on the other hand, the most exciting drug on the horizon is compactin, which blocks cholesterol synthesis. The answer is that even though the diet may push over the brink into hypercholesterolemia, most of the cholesterol in the body is derived from endogenous systhesis - a fact that was first demonstrated by Jean Wilson 15 years ago (31).

Figure 29 summarizes the daily sterol balance of the typical American. He consumes about 800 mg of cholesterol, of which he absorbs about 40% or 320 mg. But his body synthesizes about 1000 mg. of cholesterol in addition. Thus, the total sterol balance is 1320 mg/day, of which 25% is derived from the diet and 75% from endogenous synthesis. If we can reduce the endogenous synthesis by only 50%, to 500 mg per day we will have more than compensated for the 320 mg dietary input. It is clear that compactin can achieve this. Our typical American may be able to have his roast beef and eat it too!

Daily Sterol Balance in An Average American

Cholesterol Derived from Diet (800 mg cholesterol intake X 40% absorption)	= 320 mg.
Cholesterol Synthesized in the body	= 1000 mg.
Total Cholesterol Input	= 1320 mg.

Additional Approaches on the Horizon

There are several other approaches to the treatment of hypercholesterolemia that appear promising; time does not permit us to discuss them this morning, but they are listed here for reference.

1) Plasmaphoresis using a continuous flow cell separator. This technique is cumbersome and expensive but it works. When repeated at 3-week intervals it can keep the serum cholesterol at any desired level (32).

2) Ileal bypass. By preventing bile acid reabsorption this procedure will lower plasma cholesterol by up to 30% (33). It is definitely of use in some patients.

3) AOMA - This is a synthetic resin developed by Monsanto that blocks cholesterol absorption from the intestine. It has been extensively studied by John Dietschy, and is currently undergoing clinical trials. A combination of this drug and Compactin may be the best of all possible worlds.

4) The Pritiken diet. An extremely low fat, low-cholesterol diet that can also lower plasma cholesterol by up to 30% over a six-week period. Long-term effects are not known.

SUMMARY

With an improved understanding of atherosclerosis slowly emerging, physicians may be on the threshold of rational medical therapy for this disease. There is a real possibility that anti-platelet agents and cholesterol synthesis inhibitors may be effective in preventing the formation of atheromatous plaques.

The real problem may be to decide whom to treat. We can't put everybody on these drugs for a lifetime, and yet if we wait for symptomatic atherosclerosis to become manifest we may be too late. The real challenge may be to reliably identify patients with early atherosclerosis before symptoms begin. For such patients our new advice may be: "Take 1/2 aspirin tablet daily and one compactin before every steak meal."

At present, however, the most conservative prescription for atherosclerosis control would be: "One aspirin held firmly between the teeth at mealtime."

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