MEDICAL GRAND ROUNDS -- PARKLAND MEMORIAL HOSPITAL -- JANUARY 10, 1975

ATHEROSCLEROSIS

Michael S. Brown, M.D.

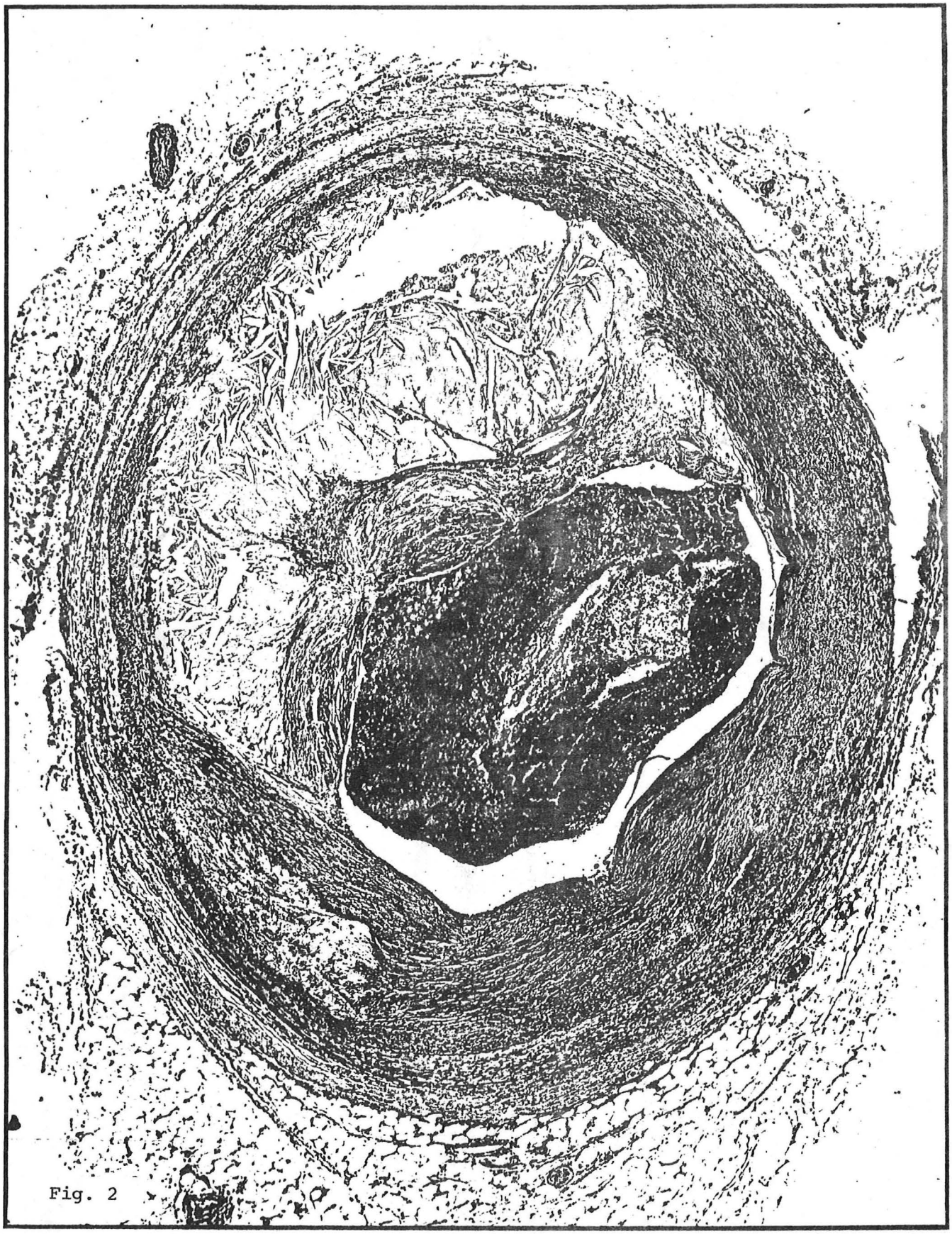


Fig. 2

The term atheroma was used in ancient Greek literature to describe a cystic space filled with amorphous material or "gruel". It was first applied to arterial pathology in the 18th century to describe a particular type of arterial placque which on sectioning exuded a yellow pultanceous material (1). Although, this grumous lesion is the most common cause of death in the United States, the approach to research in this area has been remarkably lackadaisical. In contrast to the brilliant analytical minds that have focused on the etiology of cancer, the task of unravelling atherosclerosis has until recently been left largely to a motley collection of morphologists, whose attention was fixed more on the "artery" than the science of arteriosclerosis. In the past few years, however, biochemistry, physiology and molecular biology have begun to make inroads into the pathogenesis of arteriosclerosis. This morning I will review recent advances that have led to the understanding of one major aspect of atherosclerosis -- the fatty streak.

Fig. 1 shows the wall of the ascending aorta of a 23 year old man. The most conspicuous layer is the tunica media, which consists almost entirely of smooth muscle cells. These are the most active metabolic cells in the aorta, and they have been shown to manufacture all of the contents of the interstitial connective tissue -- namely, elastin, collagen and acid mucopolysaccharides. (1a) In contrast, the tunica intima is quite thin and relatively acellular. It is covered on the luminal surface with a single layer of endothelial cells. Even though the tunica intima in the drawing is relatively thin, it is still much thicker than the aortic intima of newborn infants, which is normably only about one cell in thickness. The intimal thickening shown here is a natural process that occurs with age, particularly in the aorta and the coronary arteries. Note that the Vasa vasorum are found only in the adventitia and outer media. The intima and inner media receive all their nutrition from the lumen, which means that nutrients and oxygen have to travel a great distance before they reach the intimal cells -- a fact which may have great relevance to atherosclerosis.

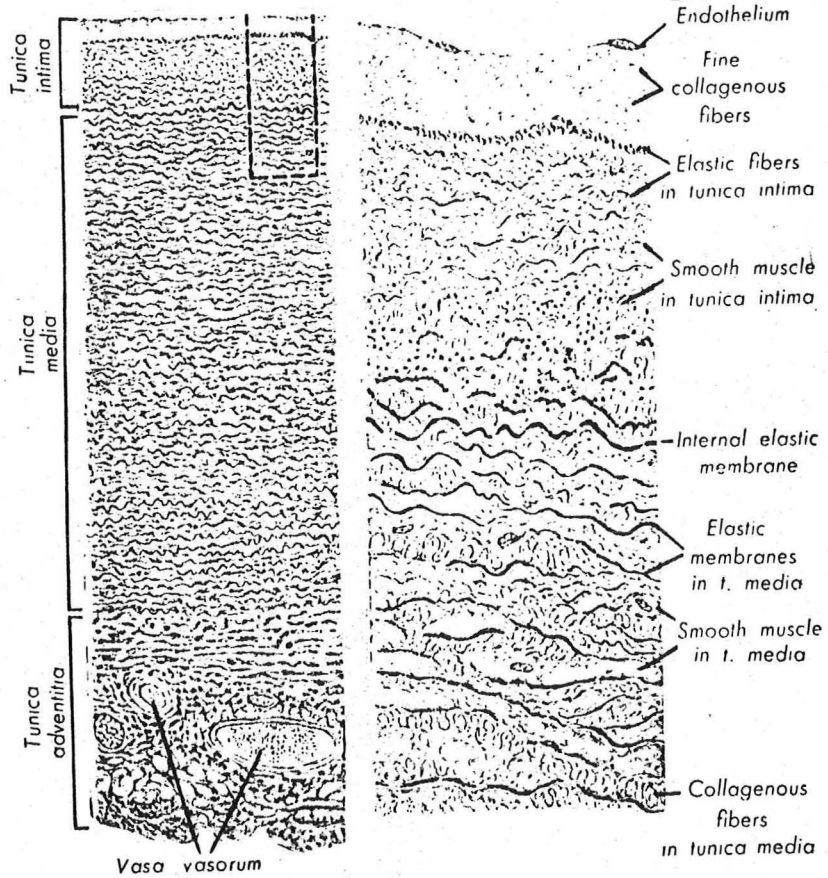


Fig.1

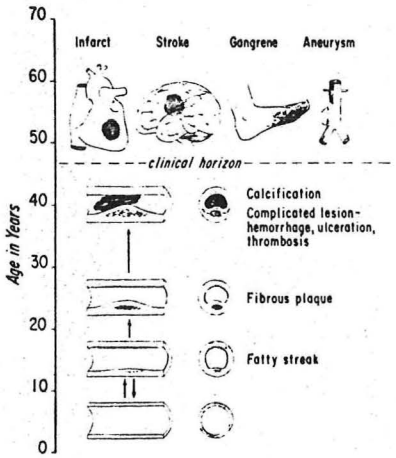


Fig. 3

Fig. 2 shows a fully developed atheromotous placque in the circumflex coronary artery of a 55 year old woman who died of a myocardial infarction. The lumen is occluded by a thrombus. The intima of the vessel is greatly thickened and severely fibrotic. The media is thinned. Deep within the intima there is a core of lipid, and many spindle shaped clear areas are seen -- the so-called cholesterol clefts. This huge amorphous mass of extracellular lipid consists almost entirely of free and esterified cholesterol. It was observations like these that first drew attention on the critical importance of cholesterol deposition in this disease. However, placques like this one are advanced and complicated; many secondary abnormalities are present. In order to study the pathogenesis of atherosclerosis we must find some way of observing the lesion before it gets to this advanced state.

In this regard great credit must go to Dr. Henry McGill and his associates. Working at the Charity Hospital in New Orleans, McGill and colleagues first focussed attention on what is believed to be the earliest lesion of atherosclerosis, the fatty streak. Fig. 3 shows an outline of the pathogenesis of atherosclerosis as postulated by McGill (2) on the basis of extensive studies of aortas and coronary arteries obtained at postmortem. The earliest lesion which they observed in subjects after the age of 10, consisted of a deposition of lipid in certain areas of the intima of the large vessels and coronaries. This lipid was observed to occur largely within smooth muscle cells that had proliferated within the intima. The initial lesion was flat, but as the age of the subjects increased two changes occurred. First, the smooth muscle cells continued to accumulate a great deal more lipid until they were converted into foam cells. Second, the lipid - containing cells became covered by a proliferation of dense fibrous tissue. Thus, the initial, fatty streak was converted into a raised fibrous placque with a deep-seated core of lipid-filled cells. Eventually, the lipid-filled cells became necrotic and their lipid was deposited as an amorphous gruel of the type shown on the previous slide. When the placques reach this size newly formed blood vessels can be seen to penetrate them from the adventitia. Frequently these blood vessels break down and memorrhage is seen into the wall of the placque. Also, the surface of the placque may ulcerate and serve as a nidus for thrombosis.

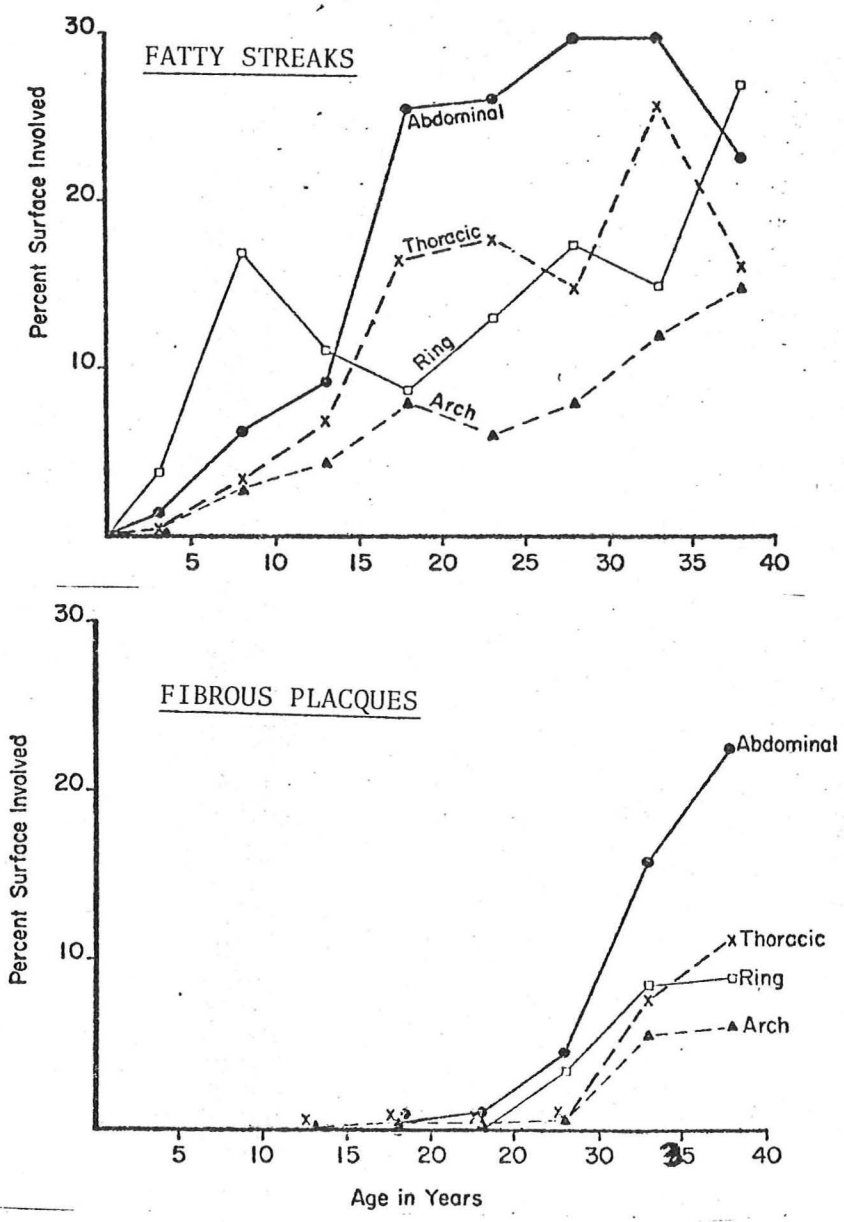
This morning, I wish to focus on the two early lesions in this process -- the fatty streak and the uncomplicated fibrous placque -- because I think we have our best chance of learning the pathogenesis of these relatively uncomplicated lesions.

Henry McGill did not discover the fatty streak -- it had been known since the turn of the century. But McGill did attempt to quantitate the occurrence of the fatty streak. To do this, these investigators developed a technique of staining intact blood vessels with Sudan IV. The uptake of this lipid stain in fatty streaks was grossly apparent and allowed, for the first time, a quantitation of the total surface area of the aorta involved with fatty streaking. Examples of the occurrence and localization of fatty streaks in coronary arteries and aortas are shown on the next four slides.

The usual histologic appearance of a Sudan -- positive fatty streak is shown in the next slide (3,4). The lipid is largely intracellular, and is contained within smooth muscle cells that appear to have proliferated in a thickened intima. There is some extracellular lipid in the form of find droplets surrounding bundles of collagen and elastin. At this state, however, there is no accumulation of dense deposits of extracellular lipid and no fibrosis. Progression of this lesion to a fibrous placque is shown on the next slide (3). The lipid is now largely extracellular and is buried beneath a dense fibrous cap. The amorphous region also contains calcium.

Evidence that the fatty streak progressed to the fibrous placque came from studies of the age of occurrence of each of these lesions (5). A striking finding was that by the age of 20, apparently healthy residents of New Orleans had more than 20 percent of their abdominal aortic surface area covered with fatty streaks (Fig. 4). Involvement was progressively less in the thoracic aorta, aortic ring and aortic arch, respectively. The relative distribution of these lesions corresponded to the relative distribution of fibrous placques, but the latter occurred on average about 15 years later. Thus, McGill and co-workers concluded that the process of atherosclerosis

FIG. 4



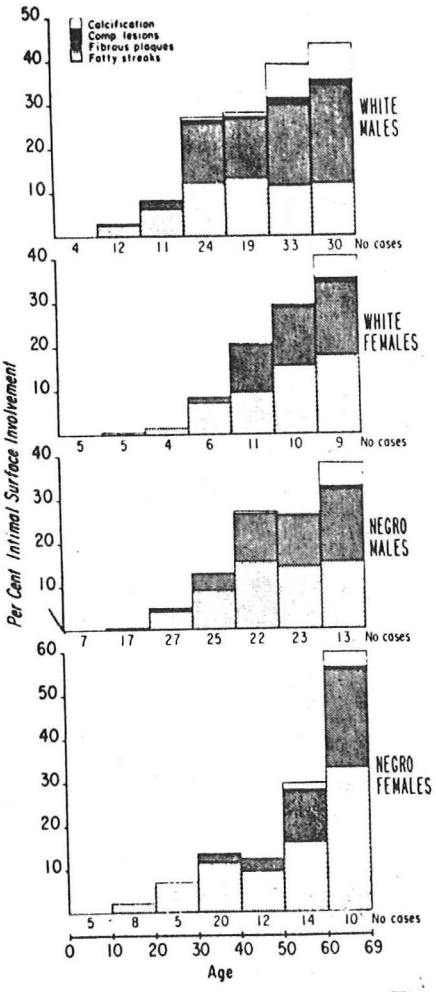


FIG. 5

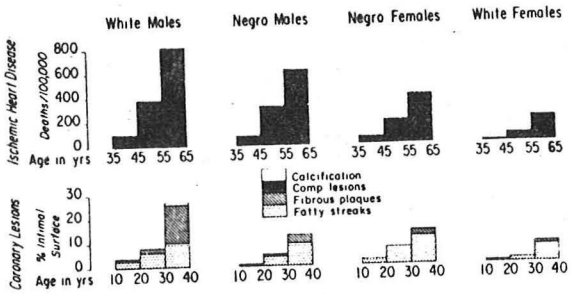


FIG. 6

begins in childhood and that it takes 15-20 years for a fatty streak to evolve into a fibrous placque. On the basis of these data there has recently been a large emphasis on so-called early prevention of atherosclerosis in childhood -- that is, dietary and drug treatment to lower the serum cholesterol level.

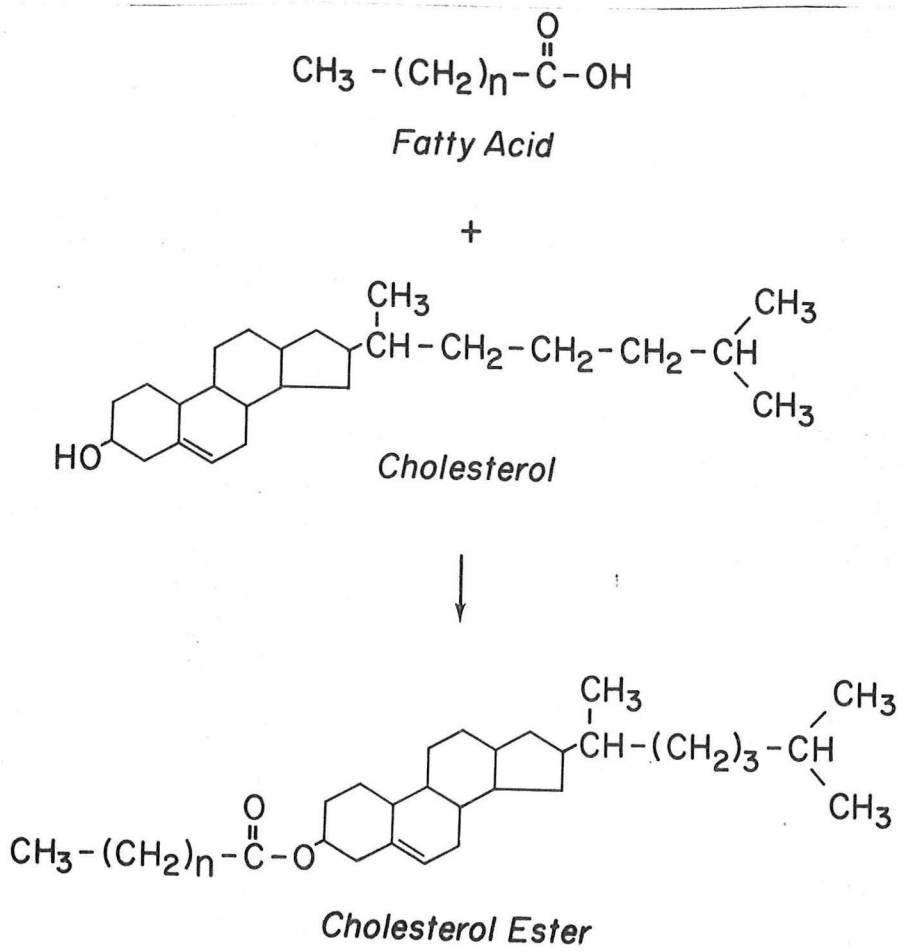
However, the evidence that juvenile fatty streaks are really the precursors of clinically significant fibrous placques is still equivocal. Thus, McGill's own data show that there is very little difference between the incidence of fatty streaks in men and women in the 30-50 year age group, whereas the incidence of ischemic heart disease is at least five times more common in women than men (Fig. 5). As shown in Fig. 6, the incidence of ischemic heart disease correlates with the occurrence of fibrous placques and not fatty streaks. Moreover, fatty streaks abound in many animal species (6) and in human populations such as the Bantu (7) that do not develop extensive fibrous placques. Thus, the theory has evolved that fatty streaking may be a "physiologic" adaptation of the aorta to increased lipid levels, and that the real pathologic process is the one that governs the conversion of the fatty streak to the fibrous placque. Alternatively, the fatty streak and the fibrous placque may be two different and unrelated lesions (1).

Perhpas the strongest evidence that the fatty streak can progress to the fibrous placque comes from work in experimental animals in which fatty streaks can be induced by feeding cholesterol, and they can be followed with time as they develop to complete fibrous placques -- as shown on the next slide.

Although the relation between fatty streaks and fibrous placques is far from settled, it is clear at least that if we understand the pathogenesis of the fatty streaks we will at least be further along toward an understanding of the fibrous placque. Accordingly, I will now review some of the recent evidence that gives insight into the sequence of events responsible in this striking lesion.

To understand this sequence we must first understand cholesterol ester metabolism. Fig. 7 shows the two forms in which cholesterol occurs in the body. Free cholesterol is an obligatory constituent of all cell membranes. On the other hand, when cholesterol is esterified to fatty acids it becomes too non-polar to exist in membranes and hence it tends to form lipid droplets, much in the same manner as triglyceride. The esterification of cholesterol appears to serve two functions in the body. First, it serves as a storage form of cholesterol, much as triglyceride serves as a storage form for fatty acids. Thus, in the adrenal gland where the storage of cholesterol is important to provide substrate for rapid steroid hormone synthesis, the cholesterol is stored as cholesterol ester. The second reason for the esterification of cholesterol is because cholesterol esters, which are liquid, appear to be much less toxic to cells than excess free cholesterol, which forms solid crystals. As pointed out by Small (8), cholesterol can dissolve in membranes only up to a 1:1 molar ratio with phospholipid. When cholesterol accumulates to levels greater than this, it precipitates out of the membrane as a solid crystal, which is probably very toxic to cells. Hence, in order

FIG. 7



to neutralize an excess of cholesterol, cells form the liquid cholesterol ester.

By the same token, nearly all cells of the body contain enzymes capable of hydrolyzing cholesterol esters. These enzymes are generally located within lysosomes, and they allow cells to hydrolyze cholesteryl esters and release cholesterol into the bloodstream.

In serum, approximately 70 percent of the cholesterol is esterified, and these esters are found in the dense hydrophobic core of lipoproteins. The two most important fatty acids that form cholesterol esters in man are oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids. In serum, the predominant acid is linoleic because serum cholesterol esters are formed by a specific enzyme, lecithin: cholesteryl acyl transferase that preferentially transfers linoleic acid from the 2-position of lecithin to cholesterol. On the other hand, in body tissues such as liver and adrenal, cholesterol oleat predominates. The unique fatty acid composition of serum cholesteryl esters has been used as a marker for whether tissues derive their cholesteryl esters directly from serum, or synthesize them de novo. Thus a high linoleic acid content in a tissue's cholesteryl esters suggests that the material is derived directly from serum without hydrolysis. On the other hand, a relatively high oleic acid content suggests that the cholesterol has been esterified in the tissues itself (9).

Why are we concerned with cholesterol esters? The answer is shown in Fig. 8, based on the data of Smith (10). In the aortic intima of children, the total lipid content is low, and there is twice as much free cholesterol as esterified. By age 65, even in normal intima there is a marked rise in total lipid content which is largely due to an increase in cholesteryl esters. This increase is similar to the one that occurs in the sclera of the eye (11,12), dura mater and tendons of the normal adult (13). Such increases are felt to represent extracellular deposition of cholesterol, possibly in the form of intact lipoproteins (11).

On the other hand, in the early fatty streak the increase in cholesteryl ester content is even more marked, and a somewhat more advanced lesion (the raised fatty nodule) shows an even more striking increase in cholesteryl esters. It is difficult to evade the conclusion that this increase in cholesteryl esters within cells is the major specific, event in the genesis of the fatty streak.

Where does this cholesterol ester come from? Table 1 summarizes the overwhelming evidence that the cholesterol moiety is derived from serum low density lipoprotein (LDL), the major cholesterol-carrying lipoprotein in plasma.

How does LDL get into the intima? The best evidence is that large particles such as LDL (200 angstroms) cannot pass between endothelial cells and instead are transported to the intima through endothelial vesicles (19). It has been calculated (albeit on the basis of the most sketchy data) that the rate of such vesicular transport is at least 10-fold greater than necessary to account for the observed accumulation of cholesterol ester in atherosclerotic intima of men (19).

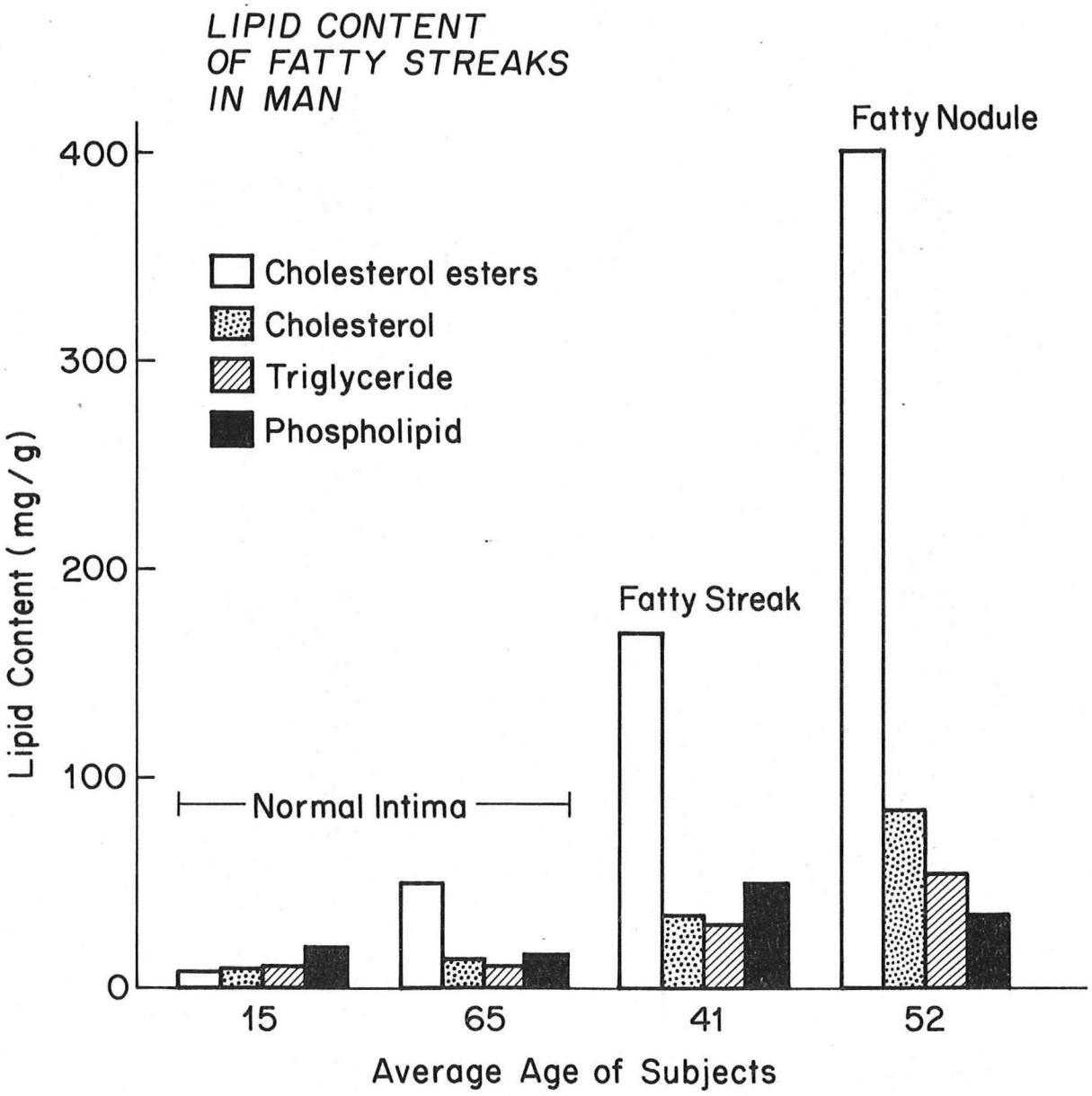

LIPID CONTENT
OF FATTY STREAKS
IN MAN
Fatty Nodule
400
300
200
100
0
Cholesterol esters
Cholesterol
Triglyceride
Phospholipid
Lipid Content (mg/g)
Fatty Streak
Normal Intima
15
65
41
52
Average Age of Subjects
Data of E. Smith

FIG. 8

TABLE 1

EVIDENCE THAT CHOLESTEROL IN ATHEROMATA
IS DERIVED FROM SERUM LDL

1. Aortic intima from animals or man (normal or atherosclerotic) synthesizes cholesterol only at a low rate (14,15,16).

2. Feeding cholesterol to animals (swine, rabbit, squirrel monkey, dog, baboon, rhesus monkey) raises serum LDL and produces cholesterol deposition in intima (6).

3. The most severe form of atherosclerosis in man (homozygous familial hypercholesterolemia) results from elevated serum LDL levels (17).

4. LDL, but not HDL, can be found in high concentrations in atheromata (18).

Evidence that LDL does indeed reach high levels in aortic intima has recently been presented by Smith (20). By the use of a new immuno-electrophoresis technique, Smith has shown that the level of LDL in normal intima is linearly related to the plasma LDL level.

How does an elevation in intimal LDL levels produce the intracellular accumulation of cholesteryl esters in smooth muscle cells that characterizes the fatty streak? Evidence indicates that this accumulation is due not merely to an uptake of intact cholesteryl esters from LDL, but that the smooth muscle cells of the intima use the LDL cholesterol to synthesize their own cholesteryl esters. The most striking evidence in this regard comes from studies of the fatty acid composition of the cholesteryl esters. As shown in Table II, the total lipid composition of the fatty streak bears a striking resemblance to the composition of LDL (9). The fatty acids of the cholesteryl esters, however, are quite different. Whereas the oleic: linoleic acid ratio in cholesteryl esters of LDL is 24:47, in the intracellular esters of the fatty streak this ratio is reversed (46:18). These data indicate that a high percentage of fatty acids are esterified to cholesterol within the cells. Similar results have been reached concerning fatty streaks of rabbits, and it was calculated that at least 50 percent of the cholesteryl ester fatty acids in this tissue arose from de novo synthesis (12).

TABLE II

COMPARISON OF
LIPID CONTENT OF LOW DENSITY LIPOPROTEINS
AND AORTIC INTIMA

PERCENTAGE OF TOTAL LIPID	NORMAL INTIMA (Age 15)	FATTY STREAK	LDL
Cholesterol Ester	12	61	59
Free Cholesterol	15	12	12
Triglyceride	34	8	10
Phospholipid	39	19	21
PERCENTAGE OF CHOLESTEROL FATTY ACIDS			
Oleic 18:1	28	46	24
Linoleic 18:2	26	18	47

Data of E. Smith

TABLE III

SYNTHESIS OF CHOLESTEROL ESTERS
IN NORMAL vs ATHEROSCLEROTIC ARTERIAL INTIMA

RATIO: $\frac{^{14}C \rightarrow \text{CHOLESTEROL ESTERS}}{^{14}C \rightarrow \text{PHOSPHOLIPIDS}}$

REFERENCE	SPECIES	NORMAL	ATHEROSCLEROTIC	FOLD CHANGE
22)	Human	.128	.421	3.3
15)	Human	.066	.157	2.4
23)	Squirrel Monkey	.028	.092	3.3
24)	Pigeon	.058	.298	5.1
25)	Rabbit	.050	.913	18.0
26)	Rabbit (Foam Cells)	--	1.08	--
27)	Rabbit (Fed Cholesterol 3 days)	.029	.081	2.8
28)	Rabbit (Endothelial Traum)	.039	.135	3.5

Direct demonstration that fatty intima is capable of esterifying cholesterol has come from several laboratories. In Table III, several of these studies are summarized. In each of these studies normal and atherosclerotic aorta were either perfused or incubated with either ^{14}C-acetate or ^{14}C-oleate and the rate of incorporation of fatty acids into cellular lipids was measured. In order to compare results from different laboratories, I have expressed the data as a ratio of the incorporation of radioactivity into the fatty acids of cholesteryl esters divided by the incorporation into phospholipids (no consistent charge is seen in phospholipid synthesis with atherosclerosis). In each of the 4 species studied, and despite wide differences in techniques, the conclusion was always the same -- namely, that the occurrence of the fatty streak was associated with a marked enhancement of cholesteryl ester synthesis in the intima. This was true in spontaneous atherosclerosis in man. It was also true when hypercholesterolemia and atherosclerosis was induced by feeding cholesterol to quirrel monkeys, pigeons and rabbits. Most interestingly, a similar increase in esterification was observed in rabbits in whom atherosclerosis was invoked not by producing hypercholesterolemia, but by placing a catheter chronically in the aorta to damage the endothelium and permit a continual leakage of lipoprotein into the intime.

Considered together the previous data indicate that increasing the LDL levels in the intima is followed by a stimulation of the rate of esterification of cholesterol by smooth muscle cells. Some insight for the mechanism of this effect has arisen from studies of cholesterol metabolism in cultured cells that Dr. Goldstein and I have been performing. Working with cultured fibroblasts we have been able to define, on the surface of the cell, a receptor molecule that specifically binds LDL. Recently, we have observed that binding of LDL to this receptor molecule is associated with a marked enhancement of the ability of the cells to incorporate fatty acids into cholesterol esters (29). Fig. 9 shows the results of one such experiment. Normal human fibro-

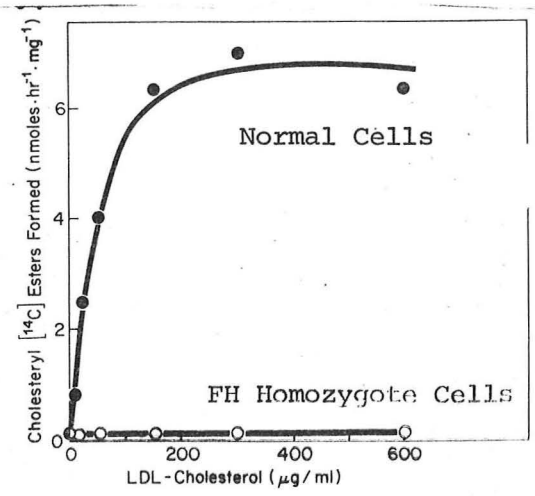


Fig. 9

blasts were grown in medium devoid of lipoproteins. Subsequently, increasing concentrations of LDL were added and the rate of incorporation of exogenous ^{14}C-oleate into cellular cholesterol esters was measured. In the absence of LDL there was no detectable cholesterol esterification. As the concentration of LDL rose the ability of the cell to synthesize cholesterol esters rose by more 40-fold, until all the LDL receptors were saturated and the process reached a maximum. The specificity of this process was shown by the observation that HDL did not stimulate esterification, even when added to the culture medium at high concentrations. The stimulation of cholesterol esterification in the presence of LDL produced a 7-fold increase in the concentration of cholesterol esters within the cell (Fig. 10) (30). Further experiments indicate that, as in the atherosclerotic smooth muscle cell, this increase involves a disproportionate increase in cholesteryl oleate, as opposed to the cholesteryl linoleate that predominates in LDL itself. HDL, which did not stimulate esterification, did not cause an increase in cholesteryl esters within the cell (30).

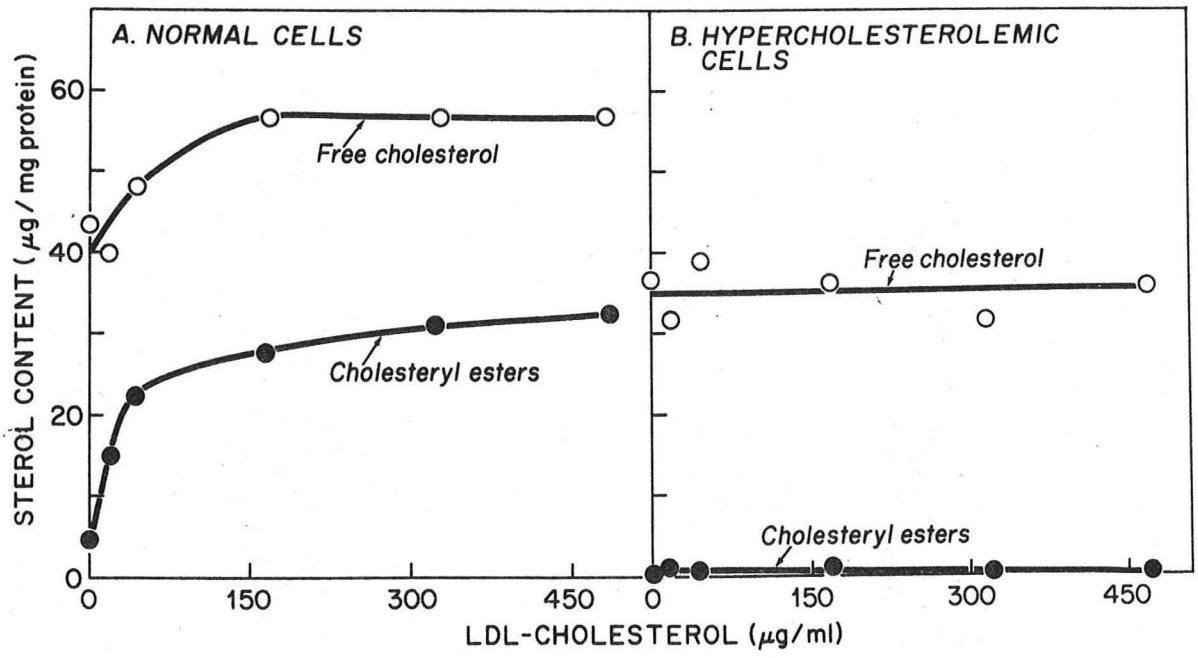


FIG. 10

As already mentioned, the predominant cell that accumulates lipid in the fatty streak is derived from the smooth muscle cell. Recently, we have had the opportunity to study cholesterol metabolism in such cells derived from the aortic media of a human fetus. Fig. 11 shows that this cell behaves identically with the fibroblast in that incubation with LDL leads to a marked enhancement of the ability of the cell to form cholesteryl esters (31).

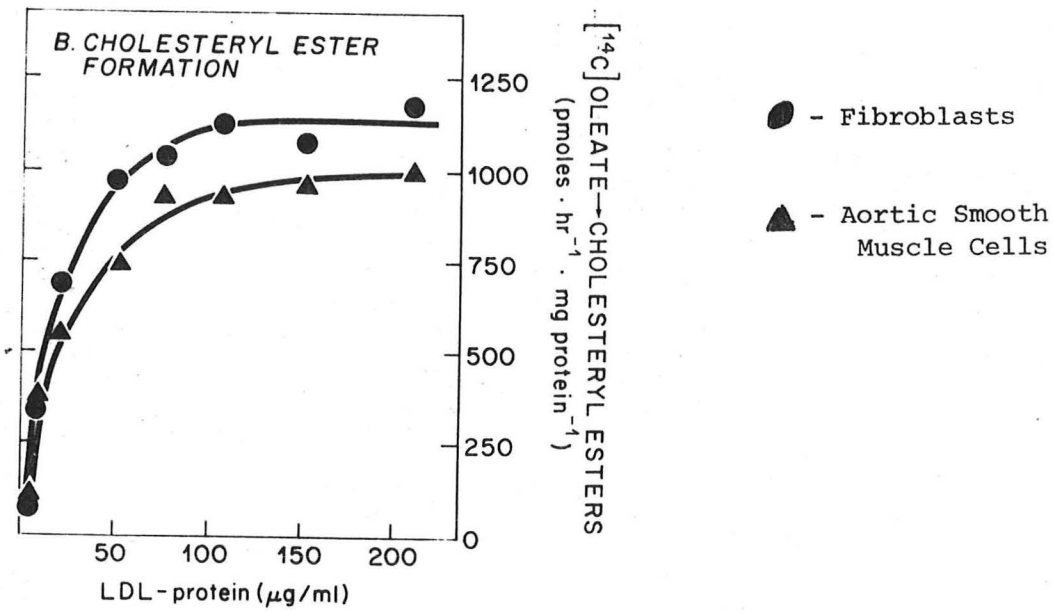


FIG. 11

We conclude from these data that the increase in cellular cholesteryl esters in the fatty streak involves a normal response of cells when they are exposed to elevated levels of LDL. It should be noted that in the culture system the concentration of LDL that causes a stimulation of ester formation is much less than the normal plasma level of LDL. We postulate that the high affinity receptor is necessary because cells in the intima as well as elsewhere, are normally exposed only to low concentrations of LDL, since the endothelial barrier only allows low levels of LDL to pass through into the intima. When cells are exposed to a higher-than-normal level of LDL, due either to an elevated LDL level in the plasma, or to an increased permeability of the endothelium, more LDL can bind to the receptor, and esterification is stimulated to a point where it outstrips the ability of cholesterol exterase to hydrolyze the esters, and cholesteryl esters accumulate in the cell.

The balance between serum LDL levels and endothelial permeability is shown diagramatically in Fig. 12. If one assumes that the concentration of LDL in the intima is linearly proportional to the serum LDL level, as the preliminary evidence indicates (20), one can see that the relation between the intimal concentration and serum LDL level will differ in different regions of the aorta depending on the local permeability to LDL. If one also assumes that a higher LDL level for a short time is as effective as a longer LDL level for a long time in producing atherosclerosis (6), then one can draw lines indicating a hypothetical threshold for atherosclerosis. It can then be seen that each area of the blood vessel will have a different propensity to develop atherosclerosis, based on both the serum LDL level and the local permeability.

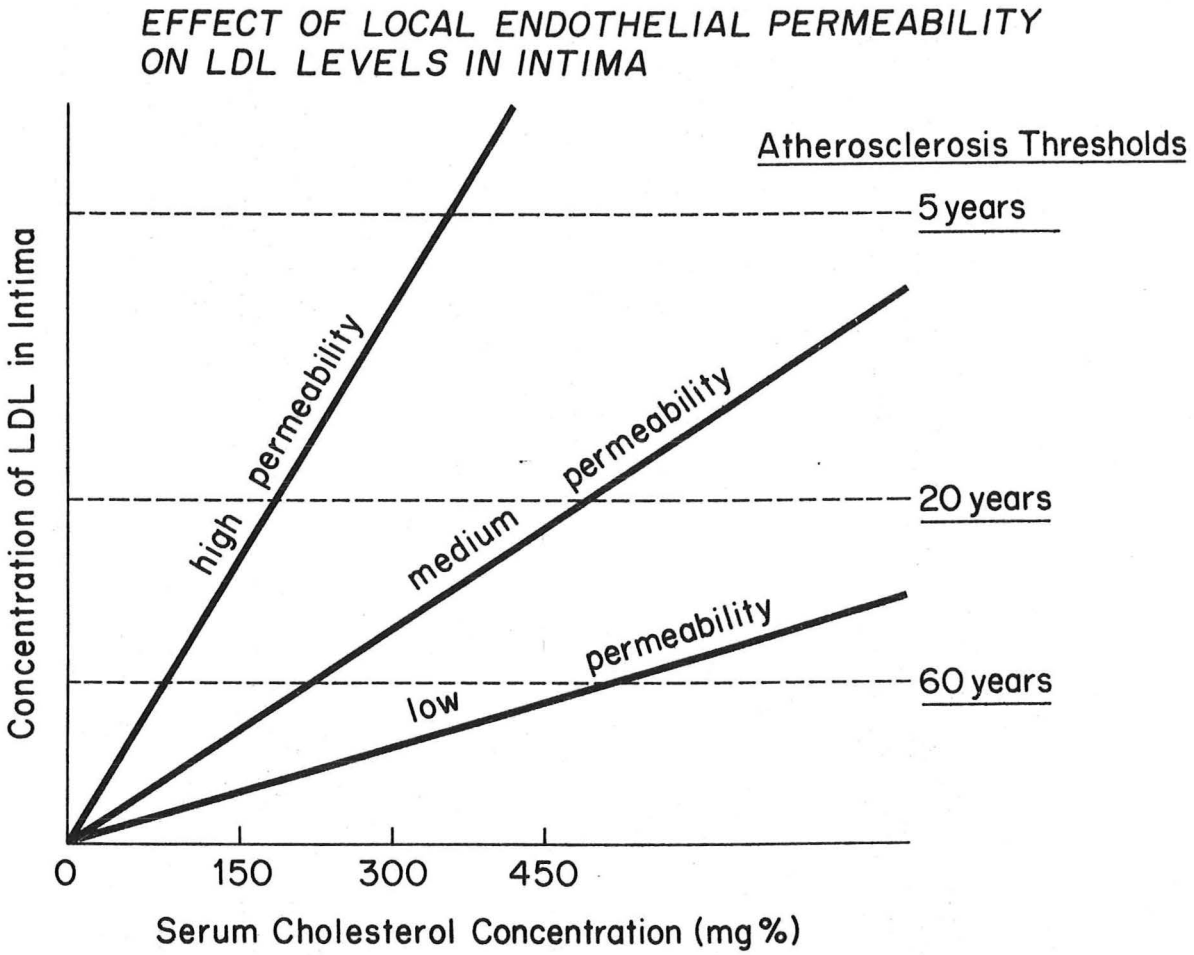

EFFECT OF LOCAL ENDOTHELIAL PERMEABILITY
ON LDL LEVELS IN INTIMA
Atherosclerosis Thresholds
5 years
20 years
60 years
high permeability
medium permeability
low permeability
Concentration of LDL in Intima
0
150
300
450
Serum Cholesterol Concentration (mg%)

FIG. 12

Do regional differences in permeability to LDL exist in blood vessels, and does this account for the tendency of the lesions to occur at certain focal sites? An affirmative answer to both of these questions is being supplied by a group of scientists at the NIH, under the direction of Donald Fry. Using both hypercholesterolemic dogs and normolipemic and hyperlipemic swine, these investigators have studied the detailed topography of aortic intimal lesions, both fibrous and fat-filled (32). These workers noted that early intimal lesions tended to occur at the orifices of major branches of the aorta. When the histology of these lesions was investigated it was seen that in normolipemic animals these lesions were the sites of intense fibrous thickening of the intima, as though they were being opposed to increased stress. When animals were made hypercholesterolemic, Fry and collaborators noted that intracellular and extracellular intimal lipid was first deposited in these regions of increased stress. Using Evans Blue dye conjugated to serum albumin these authors found that these areas of the aorta showed increased penetration of this macromolecule (33). Finally, by a technique of microscopy they were able to study the pattern of endothelial cells in en face preparations of aorta. The areas of increased permeability were associated with a clustering and local hyperplasia of endothelial cells. The authors calculated that the involved regions of the aorta were the sites that were exposed to the most intense shear stress as blood was diverted into branches of the major arteries, and concluded that this shear stress caused a hyperplastic reaction that was associated with increased permeability of the endothelium. When the serum LDL level was elevated, this led to deposition of lipids in the involved area, and the production of the fatty streak.

In summary, the data presented this morning indicate that fatty streaks occur frequently in human and animal aortas and coronary arteries. The lesion appears to involve the accumulation of cholesteryl esters in smooth muscle cells of the intima of the vessel, associated with proliferation. The reason for the ester accumulation may be a normal response of the cells to increased levels of LDL secondary to either: (1) Increased serum levels of LDL, or (2) an enhanced permeability of the endothelium to LDL.

The major question that remains is: Is the fatty streak the important lesion in the pathogenesis of atherosclerosis?

References

1. Schwartz, C.J. and Mitchell, J.R.A. (1962) The morphology, terminology, and pathogenesis of arterial plaques, Postgrad. Med. J. 38, 25-34.

1a. Ross, R. and Glomset, J.A. (1973) Atherosclerosis and the smooth muscle cell, Science 180, 1332-1339.

2. McGill, H.C., Jr., Geer, J.C., and Strong, J.P. (1963) Natural history of human atherosclerotic lesions in Atherosclerosis and Its Origin, Sandler, M. and Bourne, G.H. eds., New York, Academic Press.

3. McGill, H.C., Jr., Eggen, D.A., and Strong, J.P. (1965) Atherosclerotic lesions in the aorta and coronary arteries of man, in Comparative Atherosclerosis, Roberts, J.L. and Straus, R. eds., New York, Harper and Row.

4. Geer, J.C., McGill, H.C., Jr., and Strong, J.P. (1961) The five structures of human atherosclerotic lesions, Am. J. Path. 38, 263-287.

5. Holman, R.L., McGill, H.C., Jr., Strong, J.P., and Geer, J.C. (1958) The natural history of atherosclerosis, Am. J. Path. 34, 209-235.

6. Comparative Atherosclerosis (1965) Roberts, J.C. and Straus, R., eds., New York, Harper and Row.

7. Strong, J.P., Wainright, J., and McGill, H.C., Jr. (1959) Atherosclerosis in the Bantu, Circulation 20, 1118-1127.

8. Small, D.M. and Shipley, G.G. (1974) Physico-chemical basis of lipid deposition in atherosclerosis, Science 185, 222-229.

9. Smith, E.B. (1974) The relationship between plasma and tissue lipids in human atherosclerosis, Adv. Lipid Res. 12, 1-49.

10. Smith, E.B. (1965) The influence of age and atherosclerosis on the chemistry of the aortic intima, J. Atheroscler. Res. 5, 224-240.

11. Smith, E.B. and Slater, R.S. (1973) Relationship between plasma lipids and arterial tissue lipids, Nutr. Metab. 15, 17-26.

12. Brockhuyse, R.M. (1972) Lipids in tissues of the eye, Biochim. Biophys. Acta 280, 637-645.

13. Crouse, J.R., Grundy, S.M., and Ahrens, E.H., Jr. (1972) J. Clin. Invest. 51, 1292-1296.

14. Siperstein, M.D., Chaikoff, I.L., and Chernick, S.S. (1951) Significance of endogenous cholesterol in atherosclerosis: synthesis in arterial tissue, Science 113, 747-749.

15. Chobanian, A.V. and Manzur, F. (1972) Metabolism of lipid in the human fatty streak lesion, J. Lipid Res. 13, 201-206.

16. Newman, H.A.I. and Zilversmit, D.B. (1962) Quantitative aspects of cholesterol flux in rabbit atheromatous lesions, J. Biol. Chem. 237, 2078-2084.

17. Brown, M.S. and Goldstein, J.L. (1974) Familial hypercholesterolemia: Genetic, biochemical and pathophysiological considerations, Adv. Int. Med. 20, 273-296.

18. Wissler, R.W. (1974) Development of the atherosclerotic plaque, in The Myocardium: Failure and Infarction, Braunwald, E. ed., New York, H P Publishing Co., p. 155-166.

19. Stein, Y. and Stein, O. (1973) Lipid synthesis and degradation and lipoprotein transport in mammalian aorta, in Atherogenesis: Initiating Factors, Ciba Foundation Symposium 12, New York, Elsevier, 165-179.

20. Smith, E.B. and Slater, R.S. (1973) Lipids and low-density lipoproteins in intima in relation to its morphological characteristics, in Atherogenesis: ... (See #19).

21. Dayton, S. and Hashimoto, S. (1968) Origin of fatty acids in lipids of experimental rabbit atheroma, J. Atheroscler. Res. 8, 555-568.

22. Wahlqvist, M.L., Day, A.J., and Tume, R.K. (1969) Incorporation of oleic acid into lipid by foam cells in human atherosclerotic lesions, Circ. Res. 24, 123-130.

23. St. Clair, R.W., Lofland, H.B., and Clarkson, T.B. (1969) Influence of atherosclerosis on the composition, synthesis and esterification of lipids in aortas of squirrel monkeys (saimir:sciureus), J. Atheroscler. Res., 10, 193-206.

24. St. Clair, R.W., Lofland, H.B., Jr., and Clarkson, T.B. (1968) Composition and synthesis of fatty acids in atherosclerotic aortas of the pigeon, J. Lipid Res. 9, 739-747.

25. Day, A.J. and Wahlqvist, M.L. (1968) Uptake and metabolism of ^{14}C-labeled oleic acid by atherosclerotic lesions in rabbit aorta, Circ. Res. 23, 779-788.

26. Day, A.J. and Wilkinson, G.W. (1967) Incorporation of ^{14}C-labeled acetate into lipid by isolated foam cells and by atherosclerotic arterial intima, Circ. Res. 21, 593-600.

27. Day, A.J. and Proudlock, J.W. (1974) Changes in cholesterol-esterifying activity in rabbits fed cholesterol for three days, Atherosclerosis 19, 253-258.

28. Day, A.J., Bell, F.P., Moore, S., and Friedman, R. (1974) Lipid composition and metabolism of thromboatherosclerotic lesions produced by continued endothelial damage in normal rabbits, Circ. Res. 34, 467-476.

29. Goldstein, J.L., Dana, S.E., and Brown, M.S. (1974) Esterification of low density lipoprotein cholesterol in human fibroblasts and its absence in homozygous familial hypercholesterolemia, Proc. Nat. Acad, Sci. USA 71, 4288-4292.

30. Brown, M.S., Faust, J.R., and Goldstein, J.L. (1975) Role of the low density lipoprotein receptor in regulating the content of free and esterified cholesterol in human fibroblasts, J. Clin. Invest., in press.

31. Goldstein, J.L. and Brown, M.S. (1975) Lipoprotein receptors, cholesterol metabolism and atherosclerosis, Arch. Path., in press.

32. Fry, D.L. (1973) Responses of the arterial wall to certain physical factors, in Atherogenesis: ... (See #19).

33. Flaherty, J.T., Ferrans, V.J., Pierce, J.E., Carew, T.E., and Fry, D.M. (1972) Localizing factors in experimental atherosclerosis, in Atherosclerosis and Coronary Heart Disease, Likoff, W., Segal, B.L., Insull, W., and Mayer, J.H., eds., New York, Grune and Stratton, pp. 85-105.